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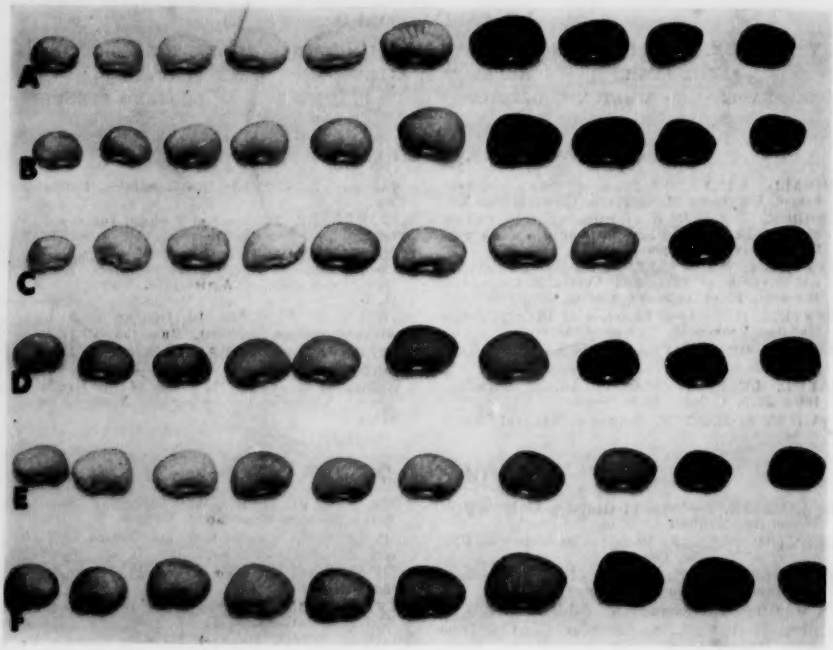

BASE SEED-COAT COLOR IN LIMA BEANS

(SEE PAGE 124)

Coffee Genetics

Curly and Angora Mink

Dimorphism in Dwarf Crawfish

New Allele at the *W*-Locus in Mice

VOLUME 48 MAY-JUNE, 1957 NUMBER 3

The American Genetic Association

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The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

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$7.00 a Year. $1.50 a copy. Prices of back numbers on application.

The

Journal of Heredity

Vol. XLVIII MAY-JUNE, 1957 No. 3

CONTENTS

Dimorphism of Chromatophore Patterns in the Dwarf Crawfish
By E. Peter Volpe and George Henry Penn 91

Pregnancy Induced in Genetically Sterile Mice
By Morris Smithberg and Meredith N. Runner 97

The Mid-South Oat Variety
By S. S. Ivanoff 101

Number of Spermatozoa Ejaculated at Copulation, Transported into the Female Tract, and Present in the Male Tract of the Golden Hamster
By M. C. Chang and Diane Sheaffer 107

Genetics of Coffea
By H. Antunes Filho and A. Carvalho 110

Evidence for a New Allele at the *W*-Locus of the Mouse
By Elizabeth S. Russell, Fay Lawson and Gretchen Schabtach 119

Inheritance of a Base Seed-Coat Color Factor in Lima Beans
By W. P. Bemis 124

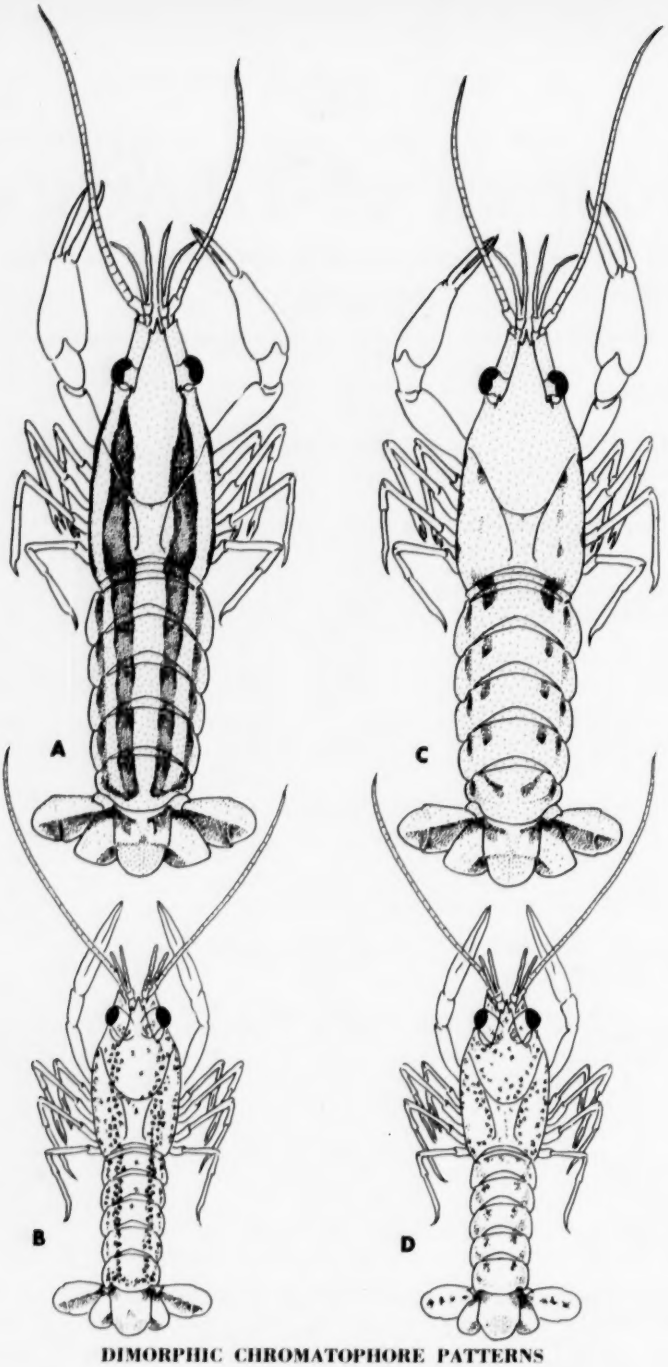
The Curly and Angora Characters in the Ranch Mink
By Richard M. Shackelford 129

Notes and Reviews

Heredity and Your Life (Rev. of A. M. Winchester: *Heredity and Your Life*)
By William M. Hexter 117

Land of the Moon-Children (Rev. of Keeler: *Land of the Moon-Children*)
By Sheldon C. Reed 127

About Mice and Man (Rev. of Avis: *About Mice and Man*)
By W. E. Heston 128



DIMORPHIC CHROMATOPHORE PATTERNS

Figure 1

Adult and third instar crawfish of the striped (*A* and *B*) and of the spotted pattern (*C* and *D*). Not to scale: third instar crawfish average 2.5 mm. carapace length; adult crawfish over 8.5 mm. carapace length.

DIMORPHISM OF CHROMATOPHORE PATTERNS IN THE DWARF CRAWFISH

E. Peter Volpe and George Henry Penn*

INDIVIDUALS of the dwarf crawfish, *Cambarellus shulfeldtii* (Faxon), possess one of two distinct chromatophore patterns (Figure 1). One is the spotted condition, in which some of the red chromatophores are aggregated into spots on the dorsal and lateral surfaces of the carapace and abdomen. The spots tend to be arranged in longitudinal rows. The other is the striped condition, which may be envisioned as a fusion of the spots to form uninterrupted bands running the length of the body. No anatomical difference can be demonstrated in crawfish exhibiting either pattern, nor is there an intermediacy or blending of the two. The striking dimorphism has been found in individuals of both sexes from populations throughout the geographic range of the species (Hobbs and Marchand[2], Penn[3,4]). Variation occurs only to the extent that the color of either pattern is changeable, depending upon the degree of dispersion of pigment granules in the red chromatophores. For example, when the pigment granules are concentrated, a certain amount of red pigment is transformed into blue pigment. The latter diffuses out of the red chromatophores into the underlying tissues, imparting a bluish tint to the pattern (for physiology of color changes, see Brown[1]).

Observations on the biology of the dwarf crawfish in eastern Louisiana by the junior author[3,4] indicated the suitability of this form for genetical investigation. Adults may be collected in large numbers at any period of the year. Spawning, although heightened at certain intervals, extends throughout the year. Samples invariably contain some ovigerous females. Of special interest is the observation that the chromatophore pattern is detectable in the second instar of the young, just prior to its release from the pleopods of the parent. The pattern is conspicuous in the free-swimming or third instar form (Figure 1).

Populations in eastern Louisiana contain different proportions of the dimorphic types, but heretofore no data were available on their relative frequencies in any one population at different time intervals. Accordingly, between April 2, 1955 and January 10, 1956, periodic collections were made from a single population in a large pond at Crown Point, Jefferson Parish, Louisiana. The relative proportion of the two patterns was found to be constant in 12 samples of the population. In addition, an examination of the young carried by females from each collection provided some insight into the mode of inheritance of the chromatophore pattern. The data permitted the formulation of a tentative inheritance scheme. Subsequently, in the summer of 1956, breeding experiments were carried out in the laboratory, the results of which reinforced the preliminary hypothesis. The difference in patterns is due to a single gene, the "striped" gene being dominant over the "spotted" allele. The analyses of the population samples and the laboratory breeding tests are presented below.

Analysis of Population Samples

The individuals from each of the 12 samples were sorted according to dorsal pattern, sex and age (adults and juveniles). Adults included all crawfish with a cephalothorax length of 8.5 mm. or greater. A length of 8.5 mm. had been recorded previously as the minimum size of ovigerous females (Penn[3]). No attempt was made to sort adult males into groups of form I and form II. Females were further divided into two categories,

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those carrying and those not carrying eggs or young.

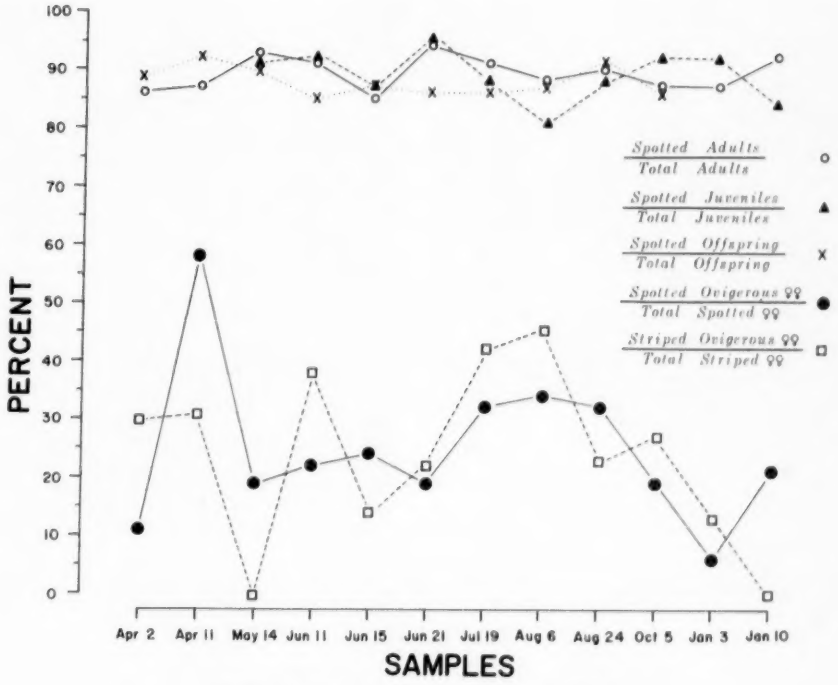
Each of the adult samples consisted predominantly of spotted forms; the proportions of spotted adults to the total number of adults in the 12 samples ranged from 85.2 to 94.2 percent (Table I, Figure 2). The differences in frequencies from one sample to the next are within the range expected by random sampling deviations. The proportion of spotted juveniles to the total juvenile population in each sample parallels closely the relative frequency of spotted adults. There is no significant difference between the mean percentages of spotted adults and spotted juveniles (89.2 ± 2.9 and 88.9 ± 4.2 respectively). Moreover, the ratio of spotted offspring recovered from ovigerous females to the total number of offspring also was constant, within limits of sampling error, from one collection date to the next (Table II, Figure 2).

The simplest interpretation of the data is that the Crown Point population is in equilibrium under the system of random mating. There is no evidence of differential mortality of the possessors of either chromatophore pattern or of preferential matings between them. The number of females bearing young varies considerably from one sample to the next (Table I). However, a proportional plot of the data (Figure 2) reveals no consistent tendency for a female of a given pigment pattern to be carrying eggs more often or less frequently than a female of the other pattern type. In the laboratory, the male of either pattern will clasp a female of the opposite pattern as readily as a female of the same pattern.

The history of the population is not known, but extensive collecting in the area has failed to disclose another population in the immediate vicinity of the Crown Point pond. The depression in which the pond lies was created in 1935 when 50 cases of dynamite were accidentally detonated on the site. The pond is a permanent body of water with a

TABLE I. Collections of *Cambarellus shulfeldtii* from Crown Point, La. April 2, 1955-January 10, 1956

Date of Collection	Spotted Individuals				Striped Individuals				Percentage of Spotted Individuals	
	Non-ovigerous females	Ovigerous females	Males	Juveniles	Non-ovigerous females	Ovigerous females	Males	Juveniles	Adults	Juveniles
April 2	59	7	12	...	7	3	3	...	85.7	...
April 11	44	60	60	...	9	4	11	...	87.2	...
May 14	50	12	61	221	2	0	9	23	91.8	90.6
June 11	70	20	75	108	5	3	8	10	91.2	91.5
June 15	107	34	130	114	18	3	26	17	85.2	87.0
June 21	170	40	180	141	14	4	6	8	94.2	94.6
July 19	108	51	129	36	11	8	8	5	91.4	87.8
August 6	65	33	70	42	6	5	11	10	88.4	80.8
August 24	77	37	107	111	10	3	12	15	89.8	88.1
October 5	82	19	96	73	8	3	19	6	86.8	92.4
January 3	88	6	106	105	13	2	16	9	86.6	92.1
January 10	52	14	62	32	6	0	5	6	92.1	84.2
Totals	972	333	1088	983	109	38	134	109		
Mean ± Standard Deviation									89.2 ± 2.9	88.9 ± 4.2



RATIOS IN 12 POPULATION SAMPLES

Figure 2

(Upper) Proportions, expressed as percentages, of crawfish with spotted pattern. (Lower) Proportions, expressed as percentages, of spotted and striped ovigerous females.

center depth of approximately 10 feet. The Crown Point population appears not to be affected by migration of individuals in or out. Adults of *Cambarellus shulfeldtii* are not known to travel overland as do members of some species of crawfish. However, in times of heavy rainfall the area around Crown Point (elevation, ± one foot above sea level) is readily flooded. A few inches of water on the ground surface may provide adequate avenues of dispersal for at least a few individuals to or from the pond.

In light of the available information, the analysis, presented below, of the patterns of the young derived from ovigerous females collected in nature and from laboratory breeding tests are premised on the assumption that the genes governing the expression of the chromatophore pattern are adaptively neutral. It is recognized that any two different genotypes are not likely to be exactly equal in adaptive value. The dimorphic color types may be maintained in nature by a balance of selective factors, the equilibrium resulting from a selective advantage of the heterozygote over both homozygotes. However, there is no compelling reason to postulate a condition of adaptive or balanced polymorphism, until it can be demonstrated that the heterozygote possesses a higher adaptive value than the homozygotes.

Offspring of Wild Females

Females carrying young from each collection were separated individually into white enamel pans containing pond water and lettuce for food. As soon as the young were released,

they were counted and examined with a stereoscopic microscope for the type of chromatophore pattern. Parents were grouped into three categories: (1) spotted females yielding only spotted offspring, (2) spotted females yielding both spotted and striped offspring, and (3) striped females yielding both spotted and striped offspring. A possible fourth category, striped females yielding only striped young, was not recovered in any of the samples. The first category was found in all samples; certain samples lacked either the second or third (Table II).

The data may be summarized as follows: 85 percent (198 of 232) of the spotted ovigerous females gave rise to spotted young only. The remaining 15 percent yielded both spotted and striped offspring. These figures may be biased. The number of offspring recovered from the spotted females that yielded only spotted forms ranged from 2 to 34, with an average of 14.5. Six or less offspring were obtained from 12 percent (24 of 198) of the spotted females that gave rise to only spotted young. Some of these spotted females may have been capable of yielding both spotted and striped types if more than six offspring had been recovered. With seven offspring, the chance that a mating was misclassified is $(\frac{1}{2})^7$ or one in 128. This calculation is based on the assumptions that the spotted pattern is recessive and striped homozygotes are rare. Then the probability of recovering all spotted offspring from a mating of a spotted female and a heterozygous striped male is $(\frac{1}{2})^n$ where n is the number of offspring. Certain statistical techniques could have been employed to correct for the low-yielding spotted females, but such refinement of the data was found not to be necessary.

A single gene difference is suggested by the data, with either the striped or spotted allele as dominant. Some indication as to which alternative is more plausible may be derived from a consideration of the Hardy-Weinberg equilibrium. Based on the figures of the adult samples, the relative frequencies of homozygous and heterozygous individuals of the dominant form may be estimated. If the spotted gene is assumed to be dominant, then in a population consisting of 89.5 percent spotted (2,393 spotted adults of 2,674), 45.7 percent

TABLE II. Inheritance of chromatophore pattern in offspring of wild females

Date of Collection	Type and Number of Parents	Type and Number of Offspring: Spotted	Striped	Mean Number of Offspring (Range in Parenthesis)	Percentage of Spotted Offspring
April 2	7 Spotted	116	0	16.6 (3-34)	89.0
	3 Striped	21	17	12.7 (8-19)	
April 11	35 Spotted	633	0	18.1 (5-30)	
	4 Spotted	59	42	25.3 (12-37)	91.7
	4 Striped	27	23	15.0 (6-18)	
May 14	7 Spotted	99	0	11.0 (7-25)	90.1
	1 Spotted	10	12	22.0 (22)	
June 11	14 Spotted	218	0	15.6 (4-30)	
	4 Spotted	44	31	18.8 (14-23)	84.7
	2 Striped	21	20	20.5 (15-26)	
June 15	18 Spotted	265	0	14.7 (3-30)	
	3 Spotted	42	32	34.7 (21-37)	86.7
	2 Striped	25	19	22.0 (21-23)	
June 21	25 Spotted	336	0	13.4 (2-24)	
	4 Spotted	36	35	17.8 (16-21)	86.1
	3 Striped	23	29	16.3 (11-23)	
July 19	29 Spotted	470	0	16.2 (2-29)	
	5 Spotted	46	50	19.2 (15-27)	86.2
	5 Striped	38	39	15.4 (8-22)	
August 6	19 Spotted	311	0	16.4 (6-31)	
	3 Spotted	31	26	19.0 (17-20)	87.1
	3 Striped	21	28	16.3 (14-20)	
August 24	32 Spotted	465	0	14.6 (6-34)	
	8 Spotted	66	37	9.9 (4-19)	90.9
	3 Striped	21	18	13.0 (6-21)	
October 5	12 Spotted	99	0	8.3 (2-20)	
	2 Spotted	9	6	7.5 (6-9)	85.9
	3 Striped	14	14	9.3 (5-18)	
Totals	198 Spotted	3012	0	14.5 (2-34)	87.8 ± 2.4
	34 Spotted	343	271	19.4 (4-37)	
	28 Striped	211	207	15.6 (5-26)	

would be homozygous spotted and 43.8 percent would be heterozygous spotted. Consequently, with random breeding, one would expect 4.8 percent of the striped females to be carrying only spotted young, 4.6 percent to be carrying both spotted and striped young, and 1.1 percent to be carrying only striped young. In other words, an ovigerous striped female collected in the field should have an approximately equal chance of bearing either all spotted or both types of young. No striped female of the 28 examined in our samples carried only spotted offspring; each carried both types. Moreover, 23.7 percent of the spotted females would be expected to carry both spotted and striped offspring. We found only 15 percent of the spotted females carrying both types of offspring. However, as noted previously, this figure possibly could have been higher if more offspring had been recovered from those spotted females from which six or less offspring were obtained.

If one assumes the striped gene to be dominant, then in a population consisting of 10.5 percent striped adults, 10.2 percent would be heterozygous striped and 0.3 percent homozygous striped. With random breeding, one would expect 0.27 percent of the spotted females to be carrying only striped young, 9.13 percent to be carrying both types of young, and 80.1 percent to be carrying only spotted young. It would be rare indeed to find either spotted or striped females yielding only striped young. No spotted females carrying only striped young were found in our samples, and all striped females gave rise to both types of offspring. Furthermore, offspring from spotted females yielding both striped and spotted young should segregate into a 1:1 ratio. This was found to be the case ($P>0.05$) among spotted females yielding both types of offspring in each sample, except for the August 24 sample ($P<0.01$). Also, we encountered a greater percentage (15 percent) of spotted females bearing both types of young than theoretically expected (9.13 percent).

The above analysis suggests a greater likelihood that the striped gene is dominant over the spotted allele. This was substantiated by the laboratory breeding tests.

Laboratory Breeding Tests

A large number of adults for breeding purposes were collected on several occasions dur-

TABLE III. Inheritance of chromatophore pattern in offspring of known parents

Exp. Set	Female Parent	Male Parent	Offspring Spotted	Offspring Striped	Probable Parental Genotypes Assuming Striped Dominant	Assuming Spotted Dominant
I.	Striped A	In Nature	08	12	A. *Ss*	A. *ss*
	Striped A	Striped B	07	17	B. *Ss*	B. ?
	Spotted C	In Nature	16	00	C. *ss*	C. *Ss*
	Spotted C	Striped B	14	10		
II.	Spotted D	In Nature	13	09	D. *ss*	D. *Ss*
	Spotted D	Striped E	08	12	E. *Ss*	E. *ss*
	Striped F	Striped E	06	15	F. *Ss*	F. ?
III.	Spotted G	In Nature	13	11	G. *ss*	G. *Ss*
	Spotted G	Spotted H	23	00	H. *ss*	H. ?
	Striped I	In Nature	14	17	I. *Ss*	I. *ss*
	Striped I	Spotted H	16	11		
IV.	Spotted J	In Nature	10	00	J. *ss*	J. *Ss*
	Spotted J	Striped K	13	08	K. *Ss*	K. *ss*
	Spotted L	In Nature	23	00	L. *ss*	L. *Ss*
	Spotted L	Striped K	12	15	M. *Ss*	M. ?
	Striped M	Striped K	09	16		
V.	Spotted N	In Nature	18	00	N. *ss*	N. *SS* or *Ss*
	Spotted N	Spotted O	20	00	O. *ss*	O. *SS* or *Ss*
	Spotted P	In Nature	15	00	P. *ss*	P. *SS* or *Ss*
	Spotted P	Spotted Q	10	00	Q. *ss*	Q. *SS* or *Ss*
	Spotted R	In Nature	17	00	R. *ss*	R. *SS* or *Ss*
	Spotted R	Spotted S	21	00	S. *ss*	S. *SS* or *Ss*
	Spotted T	In Nature	13	00	T. *ss*	T. *SS* or *Ss*
	Spotted T	Spotted U	08	00	U. *ss*	U. *SS* or *Ss*
	Spotted V	In Nature	20	00	V. *ss*	V. *SS* or *Ss*
	Spotted V	Spotted W	13	00	W. *ss*	W. *SS* or *Ss*
	Spotted X	In Nature	09	00	X. *ss*	X. *SS* or *Ss*
	Spotted X	Spotted Y	12	00	Y. *ss*	Y. *SS* or *Ss*
	Spotted Z	In Nature	26	00	Z. *ss*	Z. *SS* or *Ss*
	Spotted Z	Spotted AA	24	00	AA. *ss*	AA. *SS* or *Ss*
	Spotted BB	In Nature	21	00	BB. *ss*	BB. *SS* or *Ss*
	Spotted BB	Spotted CC	13	00	CC. *ss*	CC. *SS* or *Ss*

ing June, 1956. Fifty-eight females were separated individually into small enamel pans, into each of which a male was introduced. As a precautionary measure, a male was not placed into the pan until the female had molted, thus shedding her annulus ventralis (spermatheca). A diet of lettuce was provided, a fresh supply being added every three or four days when the pond water was changed. Gravel provided a rough floor surface to facilitate locomotion of the individuals, and *Elodea* was added in each pan to insure an adequate supply of oxygen. Females were examined each time the water was changed. Those exhibiting no apparent progress after two weeks were given another male. An effort was made to obtain successful breeding of the same female several times with different males. However, no female produced eggs more than once in the laboratory. Some males, however, were used for several fertilizations. A total of 87 matings were attempted, of which 17 (20 percent) yielded results.

The data clearly indicate that the striped condition is dominant (Table III). The experiments are grouped into five sets (I-V). An explanation of set I serves to represent the series. In set I a striped female (A) that was ovigerous at the time of collection yielded 20 young, of which eight were spotted and 12 striped. Female A, after having molted and mated with a striped male (B), gave rise to seven spotted and 17 striped offspring. Young from a spotted female (C), ovigerous when collected, possessed only the spotted pattern. Spotted female C, when mated with the aforementioned striped male B, gave rise to both spotted and striped young. The results are interpretable only on the hypothesis that striped is dominant over spotted. Striped individuals could not be recessive if a mating of the two gave rise to both spotted and striped young. The same conclusion that spotted is recessive is apparent from the data of the other sets. The mating of two striped individuals (E and F of set II) could not have resulted in both types of offspring if striped is recessive. In set III, the spotted male (H) would have had to have a different genotype for each mating if the spotted gene is dominant. In set IV, the striped female (M) must be heterozygous dominant for the striped gene. Set V consists of eight spotted females that were ovigerous when collected and each gave rise to only spotted offspring. Only spotted young were produced when each female was mated with a spotted male. If the spotted gene is dominant, it is highly likely that we would have had at least one mating between two heterozygous spotted individuals.

Offspring from the five experimental matings involving spotted and striped parents segregated into a ratio of 1:1 ($P>0.50$), as expected if the striped gene is dominant. Likewise, the three experimental matings involving striped parents resulted in a ratio not significantly different from 3:1 ($P>0.20$).

Summary

Two distinct chromatophore patterns, striped and spotted, are found in a population of the dwarf crawfish, *Cambarellus shulfeldtii*, from Crown Point, Louisiana. The bulk of the population consists of spotted individuals; the frequency of spotted forms was found to be constant in 12 samples taken at different intervals over a 9-month period. Analyses of young taken from ovigerous females collected in nature coupled with breeding tests in the laboratory revealed a single gene difference between the two patterns; the "striped" gene being dominant over the "spotted" allele.

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PREGNANCY INDUCED IN GENETICALLY STERILE MICE

MORRIS SMITHBERG AND MEREDITH N. RUNNER*

A STERILE-OBESE syndrome in mice of a colony at this laboratory has been reported by Ingalls *et al.*[5]. The female reproductive organs of these mice remain immature throughout life, although Drasher *et al.*[2] showed their reproductive organs to be responsive to gonadotropic or ovarian hormones. Runner[10] reported that ovulation occurred when homogenate of pituitary glands from obese females had been injected into prepuberal female mice. Hummel[4] transplanted ovaries from obese donors to non-obese recipients and these ovaries supported pregnancies that ultimately resulted in offspring. The above observations show that ovaries carrying homozygous *obob* genes have normal reproductive capacities. The primary cause of sexual immaturity in adult obese females therefore appears to involve the pituitary. Either there is an inadequate release of gonadotropic hormones or inactivation following their release. Conception has been reported[13] in sterile, obese females following the administration of gonadotropic hormones. Implantation, however, did not occur even though blastocysts from these obese females, when transplanted into non-obese recipients, developed into viable young. Thus the obese females produced viable ova but failed to support pregnancy, presumably because of insufficiency of pituitary gonadotropins. More recently Smithberg and Runner[14] have reported that blastocysts implant in the uteri of both intact and ovariectomized, non-obese, prepuberal females following administration of progesterone. (Gonadotropic hormones had previously brought about conception.) The minimal level of progesterone necessary to induce implantation, however, did not suffice to maintain pregnancy beyond 12 days *post coitum* (*p.c.*) Higher doses of progesterone maintained pregnancies to 19 days *p.c.* The young delivered by Caesarian section, have survived when reared by foster mothers. These mice, induced to become pregnant by administration of progesterone, were incapable of delivering their young probably because the interpubic ligament failed to lengthen and the birth canal did not enlarge. Parturition was induced by discontinuing progesterone during the last quarter of pregnancy and by administering the hormone, relaxin. Lactation followed without further treatment and some non-obese, prepuberal mothers reared their own offspring. The onset of obesity at about four weeks of age suggested that the reproductive organs of sterile, obese females may cease to develop at a level of maturity comparable to that attained by non-obese animals of the same age. The aim of the present investigation therefore was to apply, to adult obese females, hormonal therapy known to be effective in normal mice four to five weeks old and also to localize the site or sites of activity of the gene for obesity. Lane and Dickie[6] reported that some obese male mice, subjected to dietary restriction, have sired litters. However, to date, it has not been possible to correct sterility in obese females by similar regimen. In general, the sterility portion of the sterile-obese syndrome is less drastic in males than in females.

Materials and Methods

Female obese (*obob*)† mice five to 18 weeks old and ranging in body weights

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†Female mice carrying obese genes were supplied by Miss Margaret M. Dickie. Homozygous obese males (*obob*), maintained on restricted dietary intake, were supplied by Mrs. Priscilla Lane of this Laboratory.

from 23 to 59 gm. were used. Estrus and ovulation were induced by a modification of the procedure described by Runner and Gates[12]. A priming injection of pregnant mares' serum*, 3 International Units/ml. saline/animal was given intraperitoneally on the morning of the first day and about 24 hours later a second injection was given. In the late afternoon of the third day an ovulatory injection of 2 International Units of human chorionic gonadotropin was administered intraperitoneally. The females were then caged with males which were either heterozygous for obesity (*ob+*) or homozygous for obesity (*obob*), the latter having been maintained on restricted food intake. The females were observed the following morning for presence of copulatory plugs. Females failing to mate after a sequence of injections were held 10 days or more and then given a second series of treatments. Daily injections of 2.5 mg. of progesterone† in 0.1 ml. of oil were given subcutaneously beginning on days 0 (day of copulation), 1 or 2 *p.c.* in an effort to provide progestational uteri in those females which had shown copulatory plugs and continued daily until 18 days *p.c.* Some full term infants recovered by Caesarian section on the 19th or 20th day were suckled by foster mothers and kept for at least six weeks. One attempt was made to induce parturition by administering 100 Guinea Pig Units of relaxin‡ on the 13th, 16th, and the 19th day of gestation.

Results

Animals were paired for matings on 128 occasions following treatment of obese females with gonadotropic hormones. Fertilization, tubal transportation and development of embyros up to the time of implantation were consistent with observations on normal animals. The results, summarized in Table I, are based upon 82 of these pairings with non-obese (*ob+*), heterozygous males and upon 46 of these pairings with dieted, obese (*obob*) males.

Eighteen (22 percent) of the 82 obese females paired with *ob+* males had a copulatory plug the morning following ovulatory injection and subsequently these animals received progesterone daily. Observations at 19 or 20 days *p.c.* revealed females with no indications of pregnancy, 11 females with resorption sites but with no live young and five females with a total of 32 fetuses. The five mothers that carried litters near term were unquestionably obese at the onset of the experiment as judged by weight and shape of the body. The obese animals at six to 14 weeks of age weighed 23 to 40 gm. in contrast to 15 to 25 gm. for the non-obese controls of comparable age range. One of the five females was given relaxin. She delivered six live young and successfully reared two of them. Attempts were made to foster 15 of the 26 young recovered by Caesarian section and six survived long enough to be diagnosed for obesity. Thus, as the result of an effort to follow the postnatal history of 21 animals, a total of eight animals (three litters) survived long enough to show segregation of genes for obesity. The ratio of obese to non-obese animals in each litter was 1:3, 1:1, and 1:1—an overall ratio of three obese and five non-obese segregants.

The 46 pairings of obese females with homozygous, obese males (*obob* × *obob*) yielded only two (4 percent) copulatory plugs in contrast to 22 percent from the backcross type of mating. One of the females failed to maintain pregnancy. The other female, subjected to Caesarian section, provided 14 full term infants. Two of the offspring died shortly after delivery and two others died during the suckling period. All 10 of the surviving young, reared by a foster mother, became phenotypically obese. This constitutes the first report of offspring obtained from parents both of which were gentically sterile obese (*obob*).

It is noteworthy that the dieted homozygous males (*obob*) only infrequently mated with obese females. The data at hand do not permit us to determine whether the lack of libido was distributed among all obese males or restricted to relatively few. Only four percent of the *obob* males produced copulatory plugs compared to 22 percent of the *ob+* males, indicating that obese males, reduced in weight by dietary restriction, were less than 20 percent as reliable as heterozygous non-obese males.

Examination of pregnant, obese animals has revealed some of the hormonal relationships

*Pregnant mares' serum and human chorionic gonadotropin were made available through the courtesy of Dr. A. P. Grieve of Ayerst, McKenna and Harrison.

†Progesterone for this project was supplied by the Shering Corporation.

‡Relaxin (releasin) and suggestions for its use were supplied by Drs. R. L. Kroc and B. G. Steinetz of Warner-Chilcott Research Laboratories.

associated with pregnancy in mice. Corpora lutea were invariably absent; mammary glands were well developed in pregnant but not in non-pregnant mice; and the interpubic ligament elongated following treatment with relaxin. The last two observations indicated the presence of intrinsic estrogen, probably derived from the placenta. The observations suggest that these mice required a balance between estrogen (intrinsic) and progesterone (administered) to successfully support pregnancy.

Discussion

Induced pregnancies in mice, having a sterile-obese syndrome, demonstrate that viable germ cells are present in the ovary, thereby excluding the possibility of a primary sterility. Induction of ovulation in obese mice and transplanting eggs to non-obese recipients[13] also demonstrated that the sterility portion of the syndrome is a secondary one. The data presented here go further to show that when extrinsic progesterone is supplied, the reproductive tract of obese mice is capable of supporting pregnancy. The obese gene when homozygous therefore has no pleiotropic effect which precludes uterine participation in pregnancy.

Observations have indicated that in animals homozygous for the *ob* gene the reproductive tract can supply an environment compatible with the normal development of the embryo prior to and following implantation. Tubal transportation of fertilized eggs in obese mice up to 4½ months of age has confirmed findings previously reported for non-obese mice four to five weeks of age. Fertilized eggs were regularly transported to the uterus in intact and ovariectomized, prepuberal as well as in obese females[14]. Since ovariectomized animals possessed no corpora lutea, tubal transportation occurred in the absence of a source for progestational hormones. The obese mice on the other hand received 2.5 mg. of progesterone daily in addition to whatever may have been provided by their own evanescent corpora lutea. There was no indication that progesterone was a deterrent to tubal transportation.

Intrauterine enlargement of blastocysts and loss of the zona pellucida occurred regularly in uteri of intact or ovariectomized, non-obese, prepuberal females and in uteri of obese females irrespective of whether or not they received injections of progesterone. Comparable development of blastocysts has been reported for fertilized eggs transplanted to the anterior chamber of the eye[11]. Contrary to the conclusions of Corner[1] and Pincus[9], enlargement of blastocysts is not dependent upon special factors supplied either directly or indirectly from the corpus luteum.

Blastocysts in uteri of non-obese, prepuberal (intact or ovariectomized) mice that were not treated with progesterone remained unattached for as long as 22 days *p.c.*[14]. The inability of the blastocysts to attach to the uterine epithelium without extrinsic progesterone was in contrast to the blastocysts which attached to the iris in the anterior chamber of the eye. Runner[13] reported, and it was promptly confirmed by Fawcett *et. al.*[3], that fertilized eggs transplanted to the eye of castrate or intact males underwent considerable development beyond the blastocyst stage. Extra-embryonic trophoblast filled and even ruptured the eye and some differentiation of the inner cell mass occurred. Within the uterus however, in the absence of progesterone, development of the late blastocysts was arrested and a relatively steady state was maintained for more than three weeks *p.c.* Progesterone therefore seems to alter the uterine epithelium, to initiate implantation and to permit further development of the blastocyst.

It is noteworthy that in ovariectomized, non-obese, prepuberal mice as well as in obese mice, progesterone was the only extrinsic ovarian hormone required by the uterus to initiate implantation and maintenance of pregnancy. Since Lyons[7] has indicated that the ovariectomized rat requires both extrinsic estrogen and extrinsic progesterone to support the mutually interacting activities of the embryo and the uterus, we must suggest that there exists a species difference with respect to extrinsic estrogen as a prerequisite for implantation and maintenance of pregnancy.

The observation that pregnant, obese mice possessed neither corpora lutea of pregnancy nor elongated interpubic ligaments indicated that therapy with progesterone had been essential in order to circumvent the genetic sterility in obese mice. Development of mammary glands only in those animals that carried fetuses to term indicated that intrinsic hormones from the placenta (i.e. estrogens) were acting synergistically with the administered progesterone. Elongation of the inter-

TABLE I. Reproductive behavior of hormonally treated mice

Male genotype	*obob* ♀♀ treated and paired with ♂	Copulatory plugs observed	(%)	♀♀ with reabsorption sites only	♀♀ with live young	No. of live young	Phenotypic classification of young reared non-obese	obese
ob+	82	18	(22)	11	5*	32	5	3
obob	46	2	(4)	1	1	14	0	10

*One obese female was induced to deliver six young, two of which she successfully reared. All other females provided live young following Caesarian section performed on day 19 or 20 *post coitum*.

public ligament gave indication of another synergism between administered relaxin and intrinsic estrogen. Intrinsic estrogen, indicated by development of mammary glands and by synergism with relaxin, was probably derived from the placentae. These observations on corpora lutea, on interpubic ligaments, and on mammary glands demonstrate that a balance of estrogen and progesterone existed when pregnancy was successful.

The results reported here and those reported earlier[14] confirm the findings of Newton and Beck[8] that a trophic effect from the placenta influences the mammary glands and the interpubic ligament. We have extended the conclusions of Newton and Beck to indicate that estrogen, produced by the mouse placenta, can account for the "trophic influence."

The above findings provide additional information about the physiology of the *ob* gene in the female mouse. Extrinsic gonadotropins produced ovulation and conception; progesterone induced implantation of the embryos; and relaxin enabled delivery followed by suckling of the young. These corrective hormones, either directly (gonadotropins) or indirectly (progesterone, perhaps also relaxin), result from the action of the pituitary gland. We conclude that the secondary sterility associated with homozygosity for the *ob* gene is a result of the insufficiency of hormones from the pituitary.

Phenotypes of offspring resulting from induced pregnancies support previous conclusions [5,8,13] pertaining to the inheritance of obesity in this particular colony of mice. The backcross type of mating, *obob* × *ob*+, gave three obese and five non-obese offspring, a sufficiently close fit for an expected ratio of 1:1 although the numbers of animals are inadequate to verify any ratio. Offspring from the mating of *obob* × *obob* provided more critical evidence. Here 100 percent obesity (10 young) leaves no doubt that obese animals breed true to type. The crossing of two recessive homozygotes, ordinarily impossible in cases of genetically determined sterility, has been accomplished by hormonal therapy. Recessive phenotypes obtained from the presumed homozygous recessive parents suggest that the hereditary unit (*ob*) shows full penetrance and expressivity.

Both the large number of reabsorptions in treated obese females (Table I) and the low frequency of matings by obese males reduced in weight by dietary restriction make these combined therapies unsatisfactory for routine perpetuation of mice homozygous for the sterile-obese syndrome.

Summary

Hormonal therapy has offset the sterility portion of a sterile-obese syndrome in mice. Conception, implantation, maintenance of pregnancy, parturition and lactation have been the consequence of hormonal administration in these genetically sterile mice. The reactions of the obese mice to hormonal therapy were remarkably similar to those previously reported for non-obese prepuberal animals. Both failed to develop organized corpora lutea of pregnancy, both responded adequately to progesterone, both failed to develop a lengthened interpubic ligament and both responded to relaxin by delivering their young. The effectiveness of hormonal therapy supports the premise that the sterility part of the obese syndrome, at least up to 4½ months of age, is associated with reproductive organs arrested in a prepuberal condition due to insufficiency of pituitary gonadotropins. Phenotypes of the offspring, derived from induced pregnancies, augment previous data pertaining to the monogenic nature of obesity in mice of this particular colony.

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THE MID-SOUTH OAT VARIETY

A Victoria-Blight Resistant Mutant

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THE Mid-South oat variety (C.I. 6977)† is the progeny of a single homozygous seedling discovered as a Victoria-blight resistant mutant among several million susceptible individuals of the variety Victorgrain 48-93[3,5]‡. The discovery was the result of an intensive search made possible by devising, especially for this purpose, a laboratory mass screening method first described in detail in this *Journal*[4].

Since this method is to be used to differentiate Mid-South from the parent oat, a brief description is included. The method is strictly a laboratory procedure for testing oats for resistance to Victoria blight. Originally it was particularly adapted for testing large lots of seed with speed, economy of space and labor, uniformity, and accuracy, but it is equally effective with only a few seeds. Essentially, it consists of dipping the seeds in an agar culture of the fungus mixed with water in the proportion of 1 to 2, and keeping them wrapped in wet paper towels ("ragdolls") for five days at about 27°C in a moisture-saturated atmosphere. Resistant seedlings appear with three long white healthy roots and a coleoptile. Susceptible "seedlings" show only vestiges of discolored and disintegrated radicles, with or without coleoptiles. These results are made possible by the combined quick action of the invading fungus and its toxins under optimum conditions for both. The seedling reaction to infection has been either that of immunity or of complete susceptibility. No intermediate reactions have been noted when the requirements of the test have been adequately met. Recently a similar mass-screening method has been described which follows the same concepts but employs the toxins only[8,19].

Victoria blight[9,12] has been one of the major hazards to the important group of oats derived from the crown-rust resistant South American variety Victoria. This disease affects primarily the root system. The plants later exhibit aboveground symptoms of reddish striping, usually along the margins of the leaves, and finally withering of the entire plant. Depending on conditions, it may either act slowly or assume quickly an epidemic form and wipe out entire fields in a short time. At low temperatures the blight fungus seems to stunt growth but fails to induce noticeably the characteristic symptoms on the foliage. During humid and warm weather, on the other hand, the disease has often destroyed young fall-sown oats planted for early grazing. In such cases more than one replanting has been necessary to secure a stand. Later infections on the maturing crop may cause excessive lodging and reduction in grain yields. At this stage the disease may be further recognized by the black sporulation at the nodes. The epidemics of 1946-1948 have caused the elimination in some areas of many susceptible varieties from the recommended lists. In recent years the disease seems to have subsided but currently has shown itself again at various locations in the Southern states.

*From the Department of Plant Pathology and Physiology, Mississippi Agricultural Experiment Station, State College, Mississippi. Journal paper (New Series) No. 647. Credit and appreciation are due to various cooperators of the Mississippi Branch Experiment Stations for their help in performing most of the grain yield tests.

†"C.I." (Cereal Introduction) refers to accession number of the Cereal Crops Section, Field Crops Research Branch, U.S.D.A. This variety was formerly known as Mississippi HVR 41. Recently it was renamed Mid-South, prior to its release to growers in 1957.

‡Victoria blight is caused by the soil-inhabiting fungus *Helminthosporium victoriae* Meehan and Murphy.

BLIGHT-RESISTANT VS. SUSCEPTIBLE OATS

Figure 3

A—Mutant (two seedlings on left) and parent, following artificial inoculation with *H. victoriae* in the laboratory. Note destruction of roots and stunting of parent variety by the fungus. Under field conditions young stands of fall-sown oats are destroyed by this disease through the root system. *B*— Left, resistant mutant (three rows of tall plants); right, susceptible parent variety practically destroyed by the disease. Oats were grown on soil naturally infested with *H. victoriae* under conditions favorable for disease development.

Among the varieties included in the Victoria-derived group are Alamo, Arlington, Mustang, Traveler, Victorgrain 48-93, and many others[15,16]. Except for their outstanding weakness, i.e., susceptibility to Victoria blight, these oats are greatly valued for their inherent high grain-yield potential and resistance to some important races of crown-rust and other pathogens. They have been developed with great effort and expense and represent an exemplary success in oat breeding. It is for these reasons that one of them, Victorgrain 48-93, was chosen as a promising reservoir in which a blight-resistant mutant might be found; also that such a mutant might possibly possess resistance to crown rust and other diseases, in conjunction with certain agronomic qualities of high excellence.*

Description

Phenotypically, Mid-South seems indistinguishable from the parent variety Victorgrain 48-93. Its habit of growth, morphology of leaves, stems and inflorescenses, foliage color, kernel qualities, and the golden-green cast of the flowers, all seem like those of the mother variety. The ripening date has most often been the same, although at times the mutant has matured ahead of the parent. The two varieties, however, differ in two important respects—in disease reaction and in winter survival. Other blight-resistant isolates, representing rare hybrid segregants and various outcross types were also found in the screenings. Although these seemed related to the parent variety, they were thought less desirable economically, on account of greater rust susceptibility, later maturity, different kernel characteristics, or some other characteristics. Still other oats in the screenings were obvious mechanical mixtures unrelated to Victorgrain 48-93. Among the several hundred blight resistant seedlings thus found, Mid-South appeared to be the most nearly identical to Victorgrain 48-93, with no visually determinable differences, except for its Victoria blight resistance and superior winter survival.

Disease Reactions

Mid-South is highly resistant to Victoria blight and this blight resistance is its distinguishing and recommending feature (Figure 3). This resistance has been verified by repeated laboratory, greenhouse, and field tests in comparison with other oats. Some of these tests have been conducted at different localities with various strains of isolates of the blight organism, with confirming results (a distinction between this oat and the parent oat could easily be made by the laboratory method described, requiring not more than a few seeds). On the other hand, the mutant oat is less resistant to certain prevalent races of the crown-rust fungus, such as *202* and *203* (including old races 45 and 57), than the mother variety as determined by greenhouse and field tests.†

The type of rust reaction of Mid-South has been variable. At seedling stage in the greenhouse, with artificial inoculation of the primary leaf with crown rust races *202* and *203*, the type reaction has been one of "complete susceptibility." This is in contrast to the seedling reaction of the parent variety which on this primary leaf shows a necrotic lesion or blotch (Victoria resistance reaction) with minute pustules or no pustules at all. At juvenile stage (plants 6-8 weeks old) and near maturity in the field, Mid-South has shown increased resistance. The type of reaction usually recorded has been "intermediate resistant," but at times it has also been "resistant," with Victoria-type lesions, or "completely susceptible," depending on location, environmental conditions, and rust races involved (Table I and Figure 3). After a heavy infection when both Mid-South and Victorgrain 48-93 were equally covered by rust lesions or pustules, the Mid-South appears greener than its parent (Figure 4*A* and *B*). Nevertheless the rust parasite usually affects a larger foliage area on Mid-South than on Victorgrain 48-93.

To some other diseases the parent and mutant have shown similar reactions. Thus, to

*Victorgrain 48-93 was developed by the late Dr. George J. Wilds of the Coker's Pedigreed Seed Co. of Hartsville, S. C.[10]

†The crown-rust species *Puccinia coronata* (Pers) Cda. consists of an ever-increasing number of physiological races and biotypes, each race having the ability to attack some oat varieties and not others. One of the latest of these is *216*. Resistance to crown rust is relative and continually changing. It does not always protect a variety from all rust damage but gives it protection from some races of the rust[1, 2, 11, 14].

All races with a designation below 201 belong to the old classification.

stem rust (*Puccinia graminis avenae* Eriks.) both oats are rather susceptible. To loose smut (*Ustilago avenae* Pers.) they are immune to two races, but to a third race, "Old Fulghum's," both are susceptible. (Loose smut is easily and inexpensively controlled by treating the seed with a suitable fungicide.) They have shown fair resistance to culm rot, caused by a strain of *H. sativum*, Pam., King, and Bakke, to which some other oats are susceptible. To mosaic, both seem fairly resistant, and are highly resistant to powdery mildew.

Grain Yields

Mid-South was tested for grain yields, in comparison with other oats, in small replicated plots during three seasons at different locations in Mississippi and for two years at 20 or more other points in the Southern United States. It has also been given comparative "farmers' appraisals" on acre bases for yield, standing and combining abilities, uniformity of ripening, etc., in several commercial fields with favorable results. From these and other data it appears that Mid-South yields at least as much as the Victorgrain 48-93 parent (Table II). The highest yield for Mid-South in comparative tests has been 125 bu./A, and for the parent, 114 bu./A.

Cold Survival

Mid-South showed on the average greater ability to survive freezes (and the consequences of them) than the variety of its origin, or the red oat varieties commonly grown in the lower Mississippi Valley. This greater survival may be attributed not to greater foliage resistance to freezing temperatures but perhaps to the ability of the root system of the blight oat somehow to withstand invasions by fungus and other parasites following cold injury, or to the greater ability to regenerate injured organs.

CROWN-RUST REACTION ON OAT LEAVES

Figure 4

A—Mid-South natural infection on maturing oats in the field. Note Victoria-like reaction and small pustules. Leaves appeared greener than in *B*. *B*—Victorgrain 48-93, natural field infection on maturing oats. Note Victoria reaction with brown centers and hardly any pustules. *C*—Very susceptible oat, natural field infection. Dark color due to abundance of uredia and telia fruiting bodies. *D-F*—Crown-rust reaction in the greenhouse at juvenile stage, artificial inoculation with races 45 and 57, on Mid-South, Victorgrain 48-93, and susceptible oat, respectively.

Discussion

Mid-South is apparently a naturally occurring mutant of Victorgrain 48-93. It probably arose from a minor change within the hereditary structure of the parent oat, brought about by an unknown ("natural") mutagenic agent.* This change involved the gene(s) or some other hereditary determinants associated with such chemical and physiological processes that bring about resistance or susceptibility to *H. victoriae*. As a direct result of this change new characteristics arose: high resistance to the blight fungus and a lowering of resistance to two races of the crown-rust fungus. The mutant does not seem to possess the *V* factor for resistance to some races as it is found in Victoria. It should be noted, however, that the general level of rust resistance of Mid-South is considerably above that of many other oats grown, such as Delair, Taggart, (also Lee and other rust-susceptible parents of Victoria-derived varieties) even though, like them, it shows in the greenhouse a type 4 (complete susceptibility) reaction on the first seedling leaf. Mid-South represents, consequently, a new genotype combining an

*Such mutations are often referred to in the literature as "spontaneous" mutations. The term *spontaneous* should perhaps be avoided because it stresses the notion of an inner energy acting without external stimulus. In our case we cannot exclude the idea of an external albeit unknown cause inducing the mutation.

TABLE 1. Crown rust reaction of Mid-South, Victorgrain 48-93, and other oats (artificial inoculation in the greenhouse and natural infection in the field)

Year	Name of Oat	Location of trial	Age, part of plant inoculated	Crown rust race employed	Rust Reaction*	Resistance to Victoria Blight
1954	Mid-South	Greenhouse	Seedling, first leaf	202 and 203	4	
	Victorgrain 48-93	Ames, Iowa‡	Seedling, first leaf	202 and 203	0	
	Delta Red 88		Seedling, first leaf	202 and 203	4	
1953	Mid-South	Greenhouse	Juvenile, all leaves	45 and 57	2	R
	Victorgrain 48-93	State College, Miss.	Juvenile, all leaves	45 and 57	0	S
	HVR #6†		Juvenile, all leaves	45 and 57	4	R
	Delta Red 88		Juvenile, all leaves	45 and 57	4	R
1953-1956	Mid-South	Field Nursery	Adult	Probably 45 and 57	1-4 (mostly 1-2)	
	Victorgrain 48-93	McNeil, Miss.	Adult	Probably 45 and 57	0-2 (mostly 0-1)	
	HVR #6		Adult	Probably 45 and 57	4 (early and heavy)	
	Delta Red 88		Adult	Probably 45 and 57	4 (late, sporadic)	

*The following 5 classes in measuring rust reaction were employed:

0 Necrotic area with no rust pustules —very resistant type
1 Necrotic area with minute rust pustules —resistant type
2 Several minute rust pustules surrounded by chlorotic-necrotic area —medium resistant type
3 Chlorotic area with some medium-size pustles —susceptible type
4 Area covered with one to many large pustules —completely susceptible

†HVR #6 is another *H. victoriae*-resistant selection but with much lower resistance to crown rust in the greenhouse and in the field (Figure 4*C* and *F*).

‡The writer is indebted to Dr. M. D. Simons, Plant Pathologist, USDA, for performing the crown-rust tests at Ames, Iowa.

intermediate degree of Victoria-like resistance to some crown-rust races with high resistance to *H. victoriae*, and to some other pathogens. It seems that parent and offspring complement each other in some respects.

In considering the crown-rust reaction of most Victoria-derived oats, the peculiarity of the "Victoria reaction" and its physiological and economic effects on the plant should be discussed. The accepted interpretation of the nature of resistance of these oats to certain crown-rust races is based on the theory of hypersensitivity of the host to the toxins of the parasite. After a rust spore initiates an infection on a healthy leaf, the toxins produced by the fungus kill the surrounding hypersensitive cells, quickly and in advance of the penetrating parasite. Thus a barrier of dead cells is formed between the spreading fungus body and the unaffected living tissue. Since rusts are obligate parasites, unable to live on dead cells, growth soon ceases and the infection at the locus is terminated. The necrotic lesion thus formed has only a minute rust pustule at the center. A few of these lesions on a leaf are of no practical matter, but in cases of heavy and widespread infections, the leaves of these resistant oats often become "dense" with such lesions (Figure 4 *B*). By coalescing they give a burned, pathological aspect to the foliage. In such instances the damage to the plant by this Victoria-reaction effect is real. Its extent depends upon the degree of infection, temperature, and other factors.

With other rust races on the other hand, such as *216*, which is highly parasitic on Victoria at all stages of growth, and which is now spreading in Mississippi, infection results in the formation of large rust pustules (complete susceptibility), in the place of the Victoria-type necrotic lesions. Furthermore, in the field under natural infection the pustules induced by this race occur in greater numbers on the Victoria-derived oats (including Mid-South), and earlier in the season than is the case with the other races. (There are other oats of interest to the plant breeder (Santa Fe, Trispernia, Landhafer, Ukraine, etc.) which do not exhibit the hypersensitivity type of resistance or response to rust attack. These are truly resistant or immune to many crown rust races and to blight, but they are lacking in other important characteristics and are used in this country for breeding purposes only. Disease resistance alone, without other genetic factors for high-yield capacity, cold tolerance, etc., is only of partial value to the grower.)

The curious fact of type 0 lesions rendering the leaf less green than types 1 and 2 pustules covering the same size area may be again explained by the theory of hypersensitivity. The tissues of the more resistant Victorgrain 48-93 are more sensitive to the toxins of the rust fungus than are those of Mid-South. Furthermore, it is even possible that the rate

of diffusion of these toxins is in some ways more inhibited in the mutant than in the parent.

As far as any significant rust damage is concerned, however, it is the time of the rust's onset (whether early or late), and the amount of green area destroyed (whether from "hypersensitivity" reaction or otherwise), that bring down the economic impact upon the crop.

The manner of inheritance of Victoria-blight and crown-rust resistance in oats of Victoria parentage has been studied by many workers[1,6,7,12,13,17,18]. A linkage between Victoria blight susceptibility and resistance to all crown-rust races to which the variety Victoria is resistant has been repeatedly reported. It has been further suggested that the *V* factor is pleiotropic in its effects and is thus responsible for resistance and susceptibility to both diseases[6]. More recently, however, it has been brought out that Victoria and its derivatives have more than one major gene for resistance to crown rust and that the mode of inheritance, including the expression of linkage relationships, seems to depend upon the particular race involved[18]. Since no genetic studies have been conducted with the Mid-South variety by this writer, it is not possible to give here a factorial interpretation of its resistance system's *modus operandi*.

It may be postulated that the mutation wrought in the hereditary apparatus affected only one or possibly two closely linked gene pairs. This transformation has found expression in the functional activities of the root system.

Summary

A new oat, named Mid-South, is described. It originated as a Victoria-blight resistant mutant from Victorgrain 48-93, a blight-susceptible variety of Victoria origin. This new genotype was discovered in the laboratory by a mass-screening method described earlier. Phenotypically it is true to the parent. Otherwise it differs from it in disease reaction and in winter survival. To Victoria blight (*Helminthosporium victoriae*) the mutant is highly resistant or immune while the parent is completely susceptible. To those crown rust races (*Puccinia coronata*) to which Victoria is resistant, the mutant showed lower resistance than the parent. Both oats showed about the same reaction to many other diseases including resistance to two races of the loose smut (*Ustiago avenae*) and susceptibility to one race; fair to good resistance to culm rot (strain of *H. sativum*), mosaic, powdery mildew (*Erysiphe graminis* var. *avenae*), and susceptibility to stem rust (*P. graminis* var. *avenae*).

Mid-South's yield of forage is about equal to its parent. The grain yields have been on the average equal or superior.

The mutant has shown greater ability to survive freezing temperatures in the field than the parent. This is attributed not to higher cold resistance of the foliage but to the capacity of the root system to maintain itself following a freeze.

The two oats may be distinguished from one another by a simple laboratory test, based on their difference in reaction to *H. victoriae*.

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TABLE II. Comparative yields of grain in Mississippi from the Mid-South and two other oat varieties commonly grown in the state

Variety	1953-1954 (2 locations) Bu/A	1954-1955* (5 locations) Bu/A	1955-1956 (6 locations) Bu/A
Mid-South	75.5	35.3	69.4
Victorgrain 48-93	69.6	33.0	66.9
Delta Red 88	63.5	27.0	60.5

*Severe freezing injury in March, 1955, reduced grain yields by approximately one-half.

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NUMBER OF SPERMATOZOA EJACULATED AT COPULATION, TRANSPORTED INTO THE FEMALE TRACT, AND PRESENT IN THE MALE TRACT OF THE GOLDEN HAMSTER

M. C. CHANG AND DIANE SHEAFFER*

IT is a common observation that hamsters will copulate frequently in a short time. A female in heat will extend her body and elevate her pelvis when a male approaches. A male will mount her, copulate and then dismount. After a few seconds, he will copulate and dismount again and again, sometimes 20 to 30 times in half an hour. Although the insertion of the penis may be observed at copulation, whether he will ejaculate in each copulation is not known. Since the ejaculation of a large number of vigorous spermatozoa is essential to ensure fertilization, the number of spermatozoa ejaculated at copulations therefore was determined. This paper also records the number of sperm entering the female tract after a series of copulations, as well as those present in the male tract.

Animals and Procedures

Adult golden hamsters, at a body weight of about 100 gm., kept in the laboratory were used. This study was accomplished in the month of May to June, 1956. When a female was detected in heat by behavior pattern, she was placed into the cage of a male. After every five copulations, the female was held up, a glass pipette containing about 0.3 ml. of saline was inserted into the vagina, the rubber bulb of the pipette was gently pressed several times and then the washing fluid was deposited into a short test tube. This was done 3-5 times after every five copulations. The seminal plug, whenever present, was picked up and placed into the washing fluid. Under such a procedure, a male usually will copulate about 50 times in an hour. Some males may delay further copulation after about 30 times of copulation, others will continue fairly eagerly after 40 times of copulation. In order to standardize the procedure, either one hour's time or 50 times of copulation was arbitrarily used as a limitation.

The number of spermatozoa in the vaginal washing was counted four times by means of haemacytometers and the total number of sperm in each sample of vaginal washing was calculated. Immediately after the last copulation, the female was sacrificed and the vagina, both uteri and the Fallopian tubes were carefully separated and suspended in saline. The vagina and the uterus were then chopped up and the number of sperm present determined. The Fallopian tube was dissected on a slide under a steroscopic microscope and then examined under a compound microscope to determine the presence of sperm.

In the determination of the number of sperm present in the male tract, the male was either sacrificed without immediate copulation or

*Worcester Foundation for Experimental Biology, Shrewsbury, Mass. and the Department of Biology, Boston University, Boston, Mass. Supported by grants from the Josiah Macy, Jr. Foundation and the Population Council. Thanks to Dr. G. Pincus for reading the manuscript.

soon after a series of copulations. The testis was exposed, the head and the tail of epididymis were separated and the entire vas deferens was cut off. Each organ was suspended in saline and chopped up for the estimation of the number of spermatozoa.

Results

The results of this study are presented in Table I and the following points can be made from the results and other observations: (1) No sperm or very few spermatozoa was ejaculated in the first five copulations and the highest sperm count was observed within the 11th to 30th copulations, e.g., No. 3 at 11-15th and No. 2a at 26-30th copulation. From the 36th to 50th copulation, the sperm count decreased in general, although one animal (No. 2b) had a second peak at 41-45th copulation. (2) The number of copulations that a male was capable of performing is not necessarily correlated to a high sperm count, for instance, No. 4 copulated only 40 times and failed to copulate in one hour's time yet his total sperm count in the vaginal washing and that recovered from the female tract was slightly higher than that of No. 2b who copulated 50 times in half an hour (343 v.s. 258 million). (3) The number of spermatozoa present in the uteri after a series of copulations was higher than that present in the total vaginal washings (49-30 v.s. 14-112 million). This indicates that the spermatozoa may have been directly ejaculated into the uterus and that the transportation of sperm from vagina into the uterus may be very fast. The direct ejaculation of sperm into the uterus in some animals is probable because a large seminal plug was found in the uterus of one animal after being mated by No. 4. (4) The presence of a few spermatozoa in the Fallopian tubes was observed in three females examined, from 30 (No. 2b) to 60 minutes (Nos. 3 and 4) after the first copulation. This shows a rapid transportation of sperm at copulation to the site of fertilization, that is, in less than 30-60 minutes. (5) The total number of spermatozoa observed in the vaginal washings and that in the female tract was 258 and 343 million in No. 2b and No. 4 and that present in the male tract of these two animals immediately after mating was 398 and 421 million respectively. This shows that about 40-45 percent of spermatozoa has been discharged from the epididymis after a series of copulations. The two animals (Nos. 1 and 3) killed 21-26 days

TABLE I. Number of spermatozoa (in millions) ejaculated at copulation, transported into the female tract, and present in the male tract of the golden hamster

No. of copulations	No. 1	No. 2a First time	No. 2b Second time 20 days later	No. 3	No. 4
1-5	0.0	0.02	0.22	0.22	0.0
6-10	6.37	18.90*	0.05	0.04	4.98
11-15	12.42	13.97*	0.10	23.90*	0.34*
16-20	15.37	13.82*	3.87*	7.75	0.16*
21-25	9.30	20.0*	27.35*	19.80	8.18*
26-30	3.12	28.4*	15.25*	1.90	0.06
31-35	5.22	6.8*	3.92*	18.0	0.02
36-40	6.27	3.20*	17.50	2.68	0.0
41-45	.....	6.30*	25.87*	5.67	.....
46-50	.....	0.20	14.45	3.37*	.....
Total no. recovered from vaginal washings	56.07	111.61	108.58	83.33	13.74
Vagina	.....	1.3	2.38	0.12	0.05
Uteri	.....	48.8	147.35	97.7	329.65*
Tubes	.....	.....	sperm in one tube	sperm in one tube	sperm in both tubes
Total no. recovered from female tract after copulations	....	50.1	149.73	97.82	329.70
Total no. from vaginal washings and female tract	56.07	161.71	258.31	181.15	343.04
Vas.	19.37‡		20.5†	19.5‡	17.75†
Tail of Ep.	793.7		340.0	520.0	357.3
Head of Ep.	74.25		38.12	39.3	46.63
Total no. recovered from male tract	887.32		398.62	578.8	421.68

*Presence of seminal plug.
†Estimation made immediately after copulations.
‡Estimation made 21-26 days after copulations.

after mating had much larger amounts of spermatozoa in the male tract, indicating a recovery from the copulatory loss.

Discussion

In their report on the copulatory behavior of the golden hamster, Reed and Reed[4] described a "phase 1" or "unsuccessful" copulation which includes the entrance of the erected penis into the vagina and several rapid, piston-like strokes of the whole pelvic region and a "phase 2" or "successful" copulation which consists of a spasmodic last stroke and a rigidly forward movement aganist the female for a few seconds. In our observation, we were not able to observe a spasmodic reaction at copulation in the hamster as in the case of the rabbit. It was also very hard to distinguish objectively which one is unsuccessful and which one is successful according to the descriptions of Reed and Reed[4]. There was, however, a general tendency at the end of a series of copulations for the duration of intromission, the forward reaction against the female, the interval between each copulation to be prolonged.

In a study of the spermatozoa present in the male tract of rams, Chang[1] reported that the total number of sperm in the male tract was 131,834 million and that an average ejaculate of a ram contained about 4,000 million spermatozoa. This represents about a 3 percent discharge of sperm from the male tract in the ram after one copulation, whereas in the hamster, a 40-45 percent of spermatozoa was evacuated from the male tract after a series of copulations. Since a ram is hardly capable of ejaculating 15 times within one hour's time, the capacity of copulation and ejaculation or the high proportion of sperm discharged from the epididymis of the hamster may be realized.

According to Ward[6], the ovulation time of the hamster is at about 1 A. M. According to Strauss[5], it occurs on the average at 2 to 3 A. M. Since the copulation time of the present study was at about 10 P. M. and since the presence of sperm in the Fallopian tubes was observed less than 30-60 minutes after the first copulation, it seems that the spermatazoa have to wait 2 to 4 hours in the female tract for the arrival of eggs. This time interval is not simply a natural sequence because Strauss[5] has reported that when mating took place in the evening, prior to ovulation, fertilization occurred two hours after ovulation, but when mating took place in the morning, i.e., after ovulation, fertilization occurred not two hours, but four hours after mating. Since the transportation of sperm to the site of fertilization is quite fast in the hamster as observed in the present study, it seems that the hamster spermatozoa require about 2 to 4 hours in the female tract to develop their fertilizing capacity. This time interval is shorter than in the case of rabbit spermatozoa which require six hours either in the Fallopian tubes[2] or in the uterus[3] to develop their fertilizing capacity.

Summary

The number of spermatozoa ejaculated at copulation, transported into the female tract, and present in the male tract was estimated. It was found that a male hamster was capable of copulating about 50 times in an hour and that the ejaculation of sperm occurred in most of the copulations although there were only a few sperm in the first five copulations and a higher number appeared from the 11th to 30th copulation. The spermatozoa were probably directly ejaculated into the uterus and the transportation of sperm after mating to the Fallopian tubes was less than 30 to 60 minutes. The capacity for copulation and ejaculation and the proportion of sperm discharged from the male tract at mating was very high in the hamster. A 3 to 4 hour time interval for the development of fertilizing capacity of hamster spermatozoa in the female tract is deduced from these and available data in the literature.

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GENETICS OF COFFEA

XVI. The variety *typica* as a standard for genetical studies of *Coffea arabica*

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SINCE the investigations on the genetics of the coffee plant (*Coffea arabica* L.) were begun at the Instituto Agronomico, Campinas, the varieties *typica* (*C. arabica* L. var. *typica* Cramer) and *bourbon* (*C. arabica* L. var. *bourbon* (B. Rodr.) Choussy) have been used as standards for comparison. *Typica* was selected because it was the first coffee variety brought into cultivation in Brazil two centuries ago and was also the first cultivated variety in various other coffee growing countries of Latin America; in most of these it still is the predominant variety grown on commercial plantations. Furthermore, the variety *typica* is known to occur spontaneously in Ethiopia, and was the type used by Linnaeus when he described the species in 1753. The variety *bourbon* has been used in the genetical analysis of *C. arabica* because of its close resemblance to *typica* and for being widely cultivated in the State of São Paulo.

The interpretatoin of dominance relations and interactions among mutants has been somewhat hampered by the simultaneous use of two distinct standard types. For this reason the writers think it highly desirable that only the var. *typica* be considered as the wild type or standard for studies of coffee genetics. The genetic constitution of *typica* as it is known from published data and some instances of gene interactions in *Coffea arabica* are discussed in this paper.

Genetic Constitution of *Typica*

Coffea arabica differs in many respects from several other species of the genus. Instead of the common number of 22 somatic chromosomes, the great majority of the botanical and commercial varieties of *C. arabica* possess 44 somatic chromosomes[7,18]. This species is self fertile and its natural reproduction is predominantly autogamous as demonstrated with the mutant *cera* (yellow seed color)[4]. Being economically the most important species of the genus, the botanical studies of *C. arabica* have received a great deal more attention than those of any other coffee species[1,5,14,15,16,17]. Studies on the inheritance of morphological characteristics in *C. arabica* have shown that the previously described *arabica* varieties differ from *typica* by one, two, or even three main genetic factors[12,13].

A comparison with *typica* reveals that among 20 of such factors, five are dominant, six show partial dominance and nine are recessive. As a rule, the mutant gene in *arabica* induces remarkable pleiotropic changes, affecting plant size, leaf size and shape, flowers, fruits and seeds. This strong pleiotropic effect explains why many of the mutants of this species have been described (and are considered) as botanical varieties.

Table I summarizes the "wild type" alleles of *typica* and the gene changes which produced the mutants already studied. The available data for several new mutants under investigation are not sufficient to permit their inclusion in the list of standard alleles. A glance at Table I shows that only one mutant, *br*, is not associated with a variety, although it is known that *typica* usually possess dark bronze young leaves (*Br*) and that the green color of young leaves is very common among *bourbon* plants[10,21]. It must be pointed out that three well known varieties were not included in Table I. One of these is haploid (*Coffea arabica* L. var. *monosperma* Ottoländer et Cramer)[19], the second includes hexa- and octoploids (*C. arabica* L. var. *bullata* Cramer)[7] and the third

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is characterized by leaf variegation inherited through the cytoplasm (*C. arabica* L. var. *variegata*)[3,5]. The writers are presently inclined to regard such variations as forms, rather than varieties[2].

Varieties Differing from *Typica* by a Single Gene Mutation

This group includes varieties which probably originated from *typica* by a single mutation. In order to find out whether a mutant is derived from *typica* and brings the standard allele in a homozygous condition it is crossed with the *murta* variety (*Coffea arabica* L. var. *murta* Hort. ex Cramer) with the genotype *ttNana*[8,9,13]. The occurrence of 50 percent of *murta* plants in the F_1 will indicate that the mutant is homozygous *tt*. *Murta* individuals will not be found if the mutant is homozygous *TT*. The conspicuous *murta* phenotype can be easily identified in segregating progenies when the seedlings are approximately one year old.

This test indicated that the following *arabica* varieties from the Campinas collection differ from the standard type by one gene mutation: *angustifolia* (ag_1 one of two independent mutants giving this phenotype); *anomala*, (*anan*); *anormalis*, (*AmAm*); *cera* (*cece*); *erecta*, (*ErEr*); *polysperma*, (*FsFs*); *maragogipe*, (*MgMg*); *purpurascens*, (*prpr*); *goiaba*, (*sdsd*); *bourbon*, (*tt*) and *xanthocarpa*, (*xcxc*).

Varieties Differing from *Typica* by Two Mutant Characters

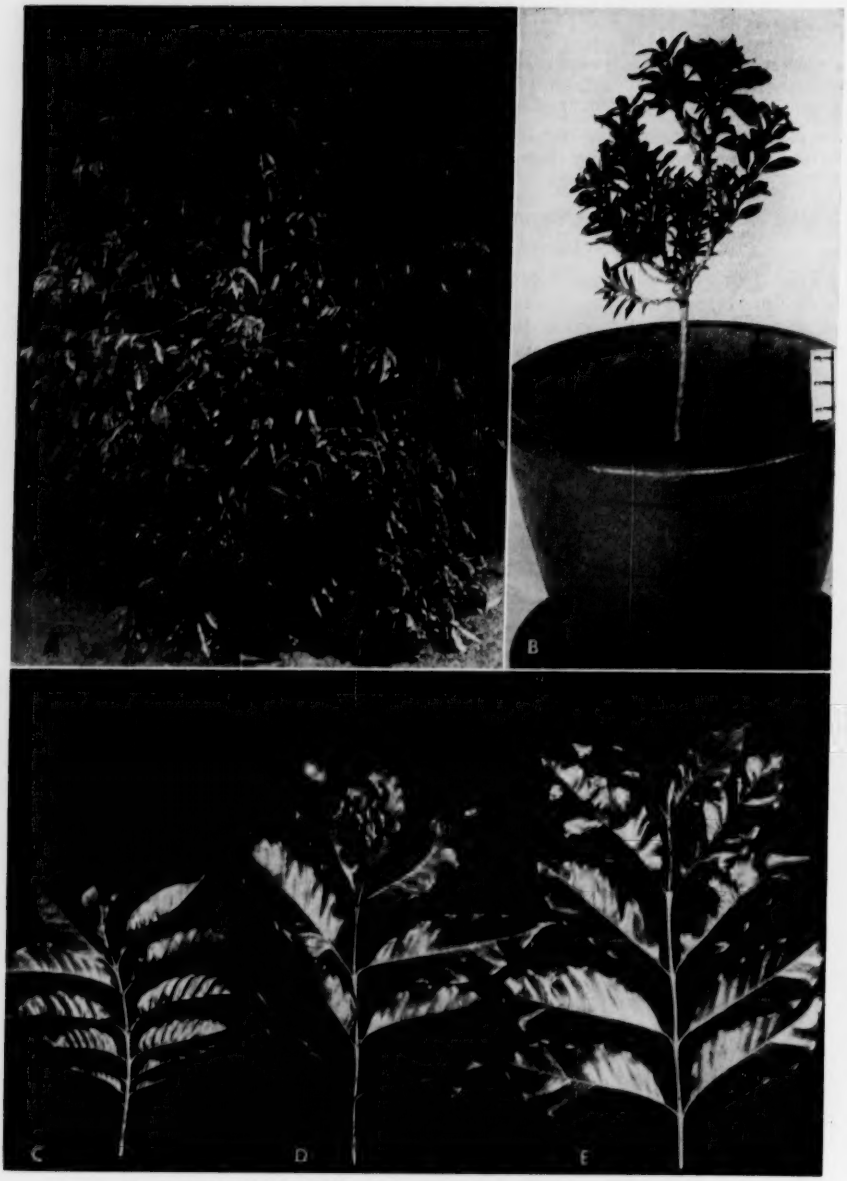
The variety *bourbon* was introduced in São Paulo in the last quarter of the past century. Being more productive than the variety *typica*, which was practically the only one grown at that time, it soon became widely used and within a few decades was being cultivated in all new coffee regions.

Several new mutants were found which probably arose from *bourbon*, their main characteristics being due to the action of a single pair of genes. As

TABLE I. Genetic constitution of *Coffea arabica* var. *typica* and of the mutants derived from *typica* by one and by two mutations*

Standard or "wild type" alleles	Varieties characterized by one gene difference from *typica*		Varieties characterized by a two gene difference from *typica*		
typica	Symbol	Variety name	Symbol		Variety name
Ag_1	ag_1	*angustifolia*			
Ag_2			(*ag*₂)	(*t*)	*angustifolia*
An	*an*	*anomala*			
am	*Am*	*anormalis*	(*Am*)	(*t*)	*anormalis*
Br†					
c	*C*	*calycanthema*			
ct			(*Ct*)	(*t*)	*caturra*
Ce	*ce*	*cera*			
cr			(*Cr*)	(*t*)	*crespa*
er	*Er*	*erecta*	*Er*	(*t*)	*erecta*
fs†	*Fs*	*polysperma*			
Lr			(*lr*)	(*t*)	*laurina*
mg	*Mg*	*maragogipe*			
Mo†	*mo*		(*mo*)	(*t*)	*mokka*‡
Na			(*na*)	(*t*)§	*nana*
Pr	*pr*	*purpurascens*			
Sd†	*sd*	*goiaba*			
Sf			(*sf*)	(*t*)	*semperflorens*
T	*t*	*bourbon*			
Xc†	*xc*	*xanthocarpa*			

*Description of these mutants is found in literature citations, 1, 2, 8, 10, 12, 13.
†Partial dominance.
‡The *mokka* variety described in Campinas[15] is *ttlrlrmomo*.
§*Na* is partially dominant in the presence of *t*.



THE STANDARD TYPE AND SOME MUTANTS SHOWING INSTANCES OF *t-na* INTERACTION

Figure 5

A—A four-year-old plant of *C. arabica* var. *typica* (standard type); *B*—plant of the *nama* variety, approximately 10 years old (*ttnama*); *C-E* shows characteristics of the leaves: *C*—*murta* (*ttNana*); *D*—*typica* (*TTNaNa*); and *E*—*bourbon* (*ttNaNa*).

this variety differs from the *typica* form by being homozygous *tt* all these new mutants depart from the standard type by two gene substitutions.

The following varieties maintained in the coffee collection at Campinas were shown to differ from the "wild type" by two main genes, one of which is the *bourbon* allele, *t; angustifolia* (ag_2ag_2); *anormalis* (*AmAm*); *caturra*, (*CtCt*); *mokka*, (*momo*); *nana*, (*nana*); *murta*, (*Nana*) *and semperflorens*, (*sfsf*).

Both the ag_1 and ag_2 alleles[13] are responsible for the *angustifolia* characteristics, i.e., narrow leaves, lesser vigor and low productivity. Conclusive evidence is available indicating that ag_1 and ag_2 are independent complementary genes, their segregation not being affected by interaction with *t*.

Similarly the *Am* mutant which characterizes the *anormalis* variety (*Coffea arabica* L. var. *anormalis* K.M.C.) shows the same phenotypic expression when it segregates in the presence of *t*. The same behavior is observed for the mutants *caturra* (*Ct*) and *semperflorens*, (*sf*). Although these two mutants were found in plants homozygous for *t* it has been verified that *Ct* and *sf* still show their characteristic effects in the presence of *T*.

Factors in Combination

Different phenotypic expressions have been observed in coffee plants when non-allelic genes are brought together in F_1 and F_2 progenies. A few cases showed almost complete epistasis, while only a partial epistasis was observed in other instances. Some gene combinations also occur with no apparent interaction, making it possible to recognize the expression of two or more mutants in a composite phenotype.

Interaction with Epistasis

Several examples of gene interaction with epistasis have already been described[9,13]. Since it is suggested here that only *typica* be considered as standard, a revision of those instances of gene interactions seems advisable, making the necessary distinction between standard and non-standard alleles. This revision is now made in accordance with the definition of epistasis proposed by Hollander[6], as being "the masking of the phenotype of a mutant or non-standard gene by that of another, not an allele." The use of the term epistasis is thus limited to gene interactions which do not involve the standard alleles of the *typica* variety; i.e., only to combinations where the expression of one of two mutants or non-standard alleles is apparent.

Interaction C-sd. Normal coffee flowers present rudimentary sepals and green ovary walls. These patterns are changed in the presence of the dominant mutant *C, calycanthema* which produces a large and conspicuous petaloid calyx[13]. Both the pedicel and ovary walls are white like the petals. Only heterozygous *Cc* plants are so far known because of an association of this character with an abnormal and non-functional pistil.

Another mutant, *sd*, is characterized by the development of a persistent calyx with five sepals[13]. The standard allele is partially dominant over this mutant.

The combination of these two mutants (*Ccsdsd*) is phenotypically identical with *calycanthema*, showing that one dose of *C* is epistatic to *sdsd*. However, the masking effect of *C* is not complete. Developed flowers from *Ccsdsd* individuals may present greenish tints in the petaloid sepals, which results from the action of the *sd* mutant.

Interaction t-na. Results from genetic analysis have shown that the differences between *typica* (Figure 5*A* and *D*) and *bourbon* depend upon a major pair of genes, *typica* being *TT*.

It had also been shown that var. *murta* (Figure 5*C*), is heterozygous *Nana*, and that *bourbon* (Figure 5*E*), is homozygous *NaNa*. The double recessive *nana* characterizes var. *nana* (dwarf plants)[8] (Figure 5*B*). It was then concluded that *Na* is incompletely dominant over *na*. This information made necessary a more complete investigation on the genetic differences between *typica* and *bourbon*, taking into consideration the genes *Tt* and *Nana*. The following segregation was observed in F_2 progenies: $\frac{1}{16}$ of *typica*, *TTNaNa*; $\frac{1}{16}$ *of bourbon, ttNaNa*; $\frac{1}{16}$ of dwarf plants, *ttnana*; $\frac{1}{16}$ of *murta, ttNana*; $\frac{1}{16}$ of a phenotype similar to *murta*, *Ttnana*, with larger leaves; $\frac{9}{16}$ of normal plants which can not be easily identified either with *bourbon* or *typica*, although clearly distinguishable from both types of *murta* and from dwarf plants

As *T* and *Na* are standard alleles it can not be stated that *T* is epistatic to *na*. It is more reasonable to say that *na* is epistatic to *t* and the segregation of the factor pair *Nana* is much less conspicuous in the presence of the standard alleles *TT* than in the presence of the mutants *tt*. Similarly, the segregation of *Tt* is less noticeable in the presence of *NaNa*, as *bourbon* has a close resemblance to *typica*. Table II summarizes this interaction.

The effects of *na* in the presence of *t* are very striking. *Nana* plants (*ttnano*) are dwarf and practically incapable of bearing fruit. *Bourbon*, (*ttNaNa*) is a commercial variety, yielding twice as much as the heterozygote *murta*, (*ttNana*)[11].

Interaction pr-br. The recessive allele *pr, purpurascens*, is responsible for the purplish

color of the plant. Although the leaves are of a deep purplish color when young, mature leaves are light purple. Flowers are pink, instead of white and the young fruits are green with a light purple pigment and dark purple streaks. This mixed color is replaced by the normal red pigment after complete ripening of the fruit.

The *Br* allele is responsible for the dark brown pigment of young leaves and is incompletely dominant to *br*. While heterozygous *Brbr* plants have light brown tips, the homozygotes *brbr* present green young leaves[10,20,21].

The F_1 from the cross *prprbrbr* × *PrPrbrbr* has green young leaves and a 3:1 ratio of green and *purpurascens* is found in F_2. As the *prpr* alleles give purple leaf color in the presence of *brbr*, it may be said that two doses of *pr* are epistatic to *br*. Unpublished data suggest that this interaction depends on the action of a third genetic factor that also affects the color of young leaves.

Combinations with Partial Epistasis

Combinations of genetic factors with partial epistasis have been observed between alleles with pleiotropic effects. Masking effects are thus limited to some characteristics, while independent expression is noticed for other traits.

Interaction lr-mo. The *lr* allele reduces plant height giving the shrub a pronounced conical shape, (Figure 6*A*). The internodes are short, the leaves are small but the flowers are of normal size. The fruits and seeds are also small, slightly pointed at the base, (Figure 6*E*). The *mo, mokka* allele determines a reduction in plant size, extremely short internodes, very small leaves with conspicuous domatias, (Figure 6*C*). The flowers are somewhat smaller than those of *typica* and the fruits and seeds are the smallest known in the *arabica* species[13], (Figure 6*F*).

The phenotype of *lrlrmomo* plants resembles that of *laurina* (*lrlr*) in size and shape, (Figure 6*B*). Such plants have extremely short internodes and very small leaves with conspicuous domatias of the *mokka* type. The flowers, fruits and seeds are also small like those of *mokka*. Plants of the *lrlrMomo* constitution are also similar to *laurina* in size and shape. The leaves are similar to those of *laurina*, except for the domatias, which are larger. Fruits and seeds, however are roundish like those of *mokka* and slightly larger. The *mo* allele is epistatic to *lrlr* in the shape of leaves, size of domatias and shape and size of fruits and seeds.

Interaction Mg-mo. The dominant allele *Mg* is responsible for an increase in size of all organs of the coffee plant, affecting plant height, length of internodes and the size of leaves, fruits and seeds. In spite of its pronounced vegetative vigor the productivity of *maragogipe* plants is low. The chromosome number is normal ($2n = 44$). The effects of the *mokka* allele *mo* were already mentioned and are to some extent opposite to those of the *Mg* allele. In plants of the *MgMgmomo* constitution, the epistatic effects of the *mo* allele can be observed in domatia size, fruit shape and particularly in seed shape. In other plant and fruit characteristics there is a combination of effects of both the *Mg* and *mo* alleles.

Interaction ag_1-lr. The individual effects of these alleles have already been mentioned. In the combination ag_1ag_1lrlr the size and shape of the plants, as well as the fruit and seed characteristics, are similar to those of *laurina*. Only the leaves are different, being of the *angustifolia* type. The epistatic effect of ag_1 is therefore restricted to leaf shape.

Combinations without Epistasis

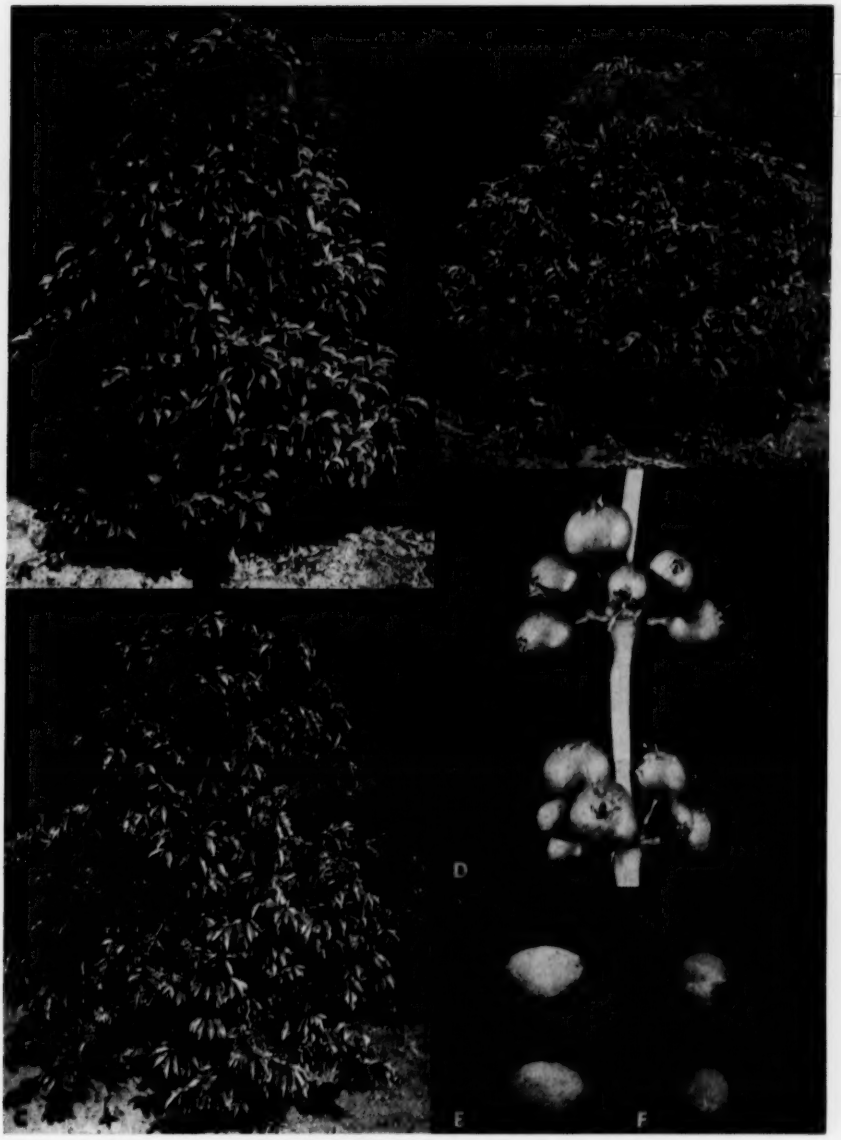
A rather large group of mutants in coffee does not show apparent epistasis but a combining effect of their expressions. Two examples will be mentioned.

Interaction pr-xc. The yellow color of the pericarp in coffee is controlled by one main pair of factors, *xanthocarpa*, (*xcxc*). The *pr* allele, as already mentioned, controls the *purpurascens* color of the leaves and also the slight *purpurascens* color of the young fruit that becomes dark red as it ripens. The pericarp of *prprxcxc* plants is still yellow but slightly darker than in homozygous *PrPrxcxc* plants. As the light purple color of the fruit, due to *pr* is not completely masked as in the presence of the standard (*Xc*), the expression of *pr* in the presence of *xc* results in the darker yellow color of the pericarp.

Interaction Fs-sd. The *Fs* allele gives an extreme fasciated phenotype and shows a pronounced pleiotropic effect. In *FsFs* plants the main stem is extremely fasciated. The flowers and fruits are also affected, the fruits being spherical and provided with many irregular seeds. The *Fs* allele shows incomplete dominance and the fruits of heterozygous plants are also round, with a smaller number of seeds. The *sd* factor, as already described, controls the development of the sepals which are large, foliaceous and persistent in *sdsd* plants and of

TABLE II. Effects of one and two gene substitutions for the mutants *t* and *na*

Standard alleles *typica*	One gene substitution	Two gene substitution
TT	*tt*—bourbon	
NaNa	*nana*—normal	*tt nana*—nana



GENE INTERACTIONS WITH PARTIAL EPISTASIS AND INDEPENDENT EXPRESSION

Figure 6

Distinctive plant characteristics are exhibited either by (*A*) *laurina* (*lrlrMoMo*), or (*C*) *mokka* (*LrLrmomo*). *Mokka* plants with the constitution *lrlrmomo* (*B*), are more similar to *laurina*. Seeds of both types of *mokka* (*F*) are roundish, while those of *laurina* (*E*) are pointed. *D*—Fruits of plants of the constitution *Fsfs Sdsd* show independent expression of both factors.

smaller size in the heterozygotes. Double heterozygotes *Fsfs Sdsd* have large, round fruits with many seeds and several persistent, foliaceous and small sepals (Figure 6*D*).

Discussion

It would be superfluous to emphasize the need of establishing a standard type for genetic analysis in *Coffea arabica*. While it might be difficult to choose a proper standard type for several other plant species, the choice is relatively easy in the case of *C. arabica*. This species is a perennial and most of its varieties are self-fertile. Besides, it is a predominantly autogamous species with approximately 10 percent of natural cross-pollination[4]. The variety *typica* presents many advantages as a standard: it was used by Linnaeus as the type of the species; its morphological characteristics are well known and their genetical analyses have been carried out to a large extent. It appears to have been the first variety to be cultivated in Arabia, Java, and in Latin America and it still is extensively cultivated in many coffee regions of Central and South America. This does not necessarily imply that *typica* represents the most primitive type of *C. arabica*, even if it is considered that this variety can still be found in Ethiopian forests. From a morphological standpoint, two varieties of *C. arabica*, both probably originated from Ethiopia, namely, *laurina* and *mokka*, are more similar to the diploid species of *Coffea* which are known to occur spontaneously in the African continent[1]. It has been difficult to establish whether or not the *bourbon* variety is more primitive than *typica*. Only an extensive morphological and cytogenetical investigation of the spontaneous forms of *C. arabica* from Ethiopia would give more precise information. A first approach in this direction was made by Sylvain[22], who recently collected several wild and cultivated types of *C. arabica* in Ethiopia. These collections are now being maintained in the coffee gardens of some countries.

The *typica* coffee plants, the genetic constitution of which is described in this paper, are maintained at the Genetics Department of the Instituto Agronomico under the numbers 10 and 12. Grafting was used to propagate the original plants, selected in an old coffee plot at the Central Experiment Station, Campinas. In order to permit a better comparison, it would be desirable that these plants or their progenies be used as a standard in other experiment stations where genetic analysis of *Coffea arabica* is being carried out. Seeds or cuttings[5] from these plants will be supplied on request.

The genetic analysis of the known varieties of *C. arabica* indicated that they usually differ from *typica* by substitution of one or two main pair of alleles, which as a rule, show marked pleiotropic effects. This perhaps explains why the majority of varieties, described as such by taxonomists unaware of their genetic relationships, correspond, to single or double mutants of var. *typica*. With some exceptions the same procedure is still adopted in Campinas in the description of new varieties. In order to be described as a new variety of *C. arabica* it is necessary to obtain the new alleles in a homozygous condition and to evaluate their effects in relation to the standard type. Besides, the phenotypical effects of new alleles must be sufficiently large so as to allow a clear phenotypical identification. Phenotypes resulting from interactions among known mutants, or similar effects of different alleles that affect the same characteristic, resulting in indistinguishable phenotypes as for example the angustifolia ag_1 and ag_3 alleles should not be considered as characterizing new varieties[2].

A clearer understanding of gene interactions is achieved when only *typica* is taken as the standard type, instead of using *bourbon* and/or *typica*. The new interpretations of epistatic relationships among some genes already known in the coffee plant, although differing from previous views, do not conflict with the available evidence on the segregations of the involved factor pairs.

Summary

In order to facilitate the understanding of dominance relations and the interpretation of interaction among the mutants found in *C. arabica*, it is proposed that *typica* be considered the only standard or "wild type" for genetic studies in this species.

Because varieties of *Coffea arabica* usually differ from *typica* by one or two main genetic factors, it is possible to group them into two classes according to their genetic constitution as related to the standard.

According to Hollander's definition of epistasis the known interactions among genes that control related characteristics in *C. arabica* is discussed.

The use of *typica* as a standard for genetic work in *Coffea arabica* allows a better understanding of dominance relations and a clearer interpretation of factor interaction among the mutants of this species.

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HEREDITY AND YOUR LIFE

THE appetite of the intelligent layman for books elucidating various scientific subjects is well attested to by the popularity of such books as Rachel Carson's *The Sea Around Us* and C. W. Ceram's *Gods, Graves and Scholars*, to mention only two. Such books have clearly demonstrated the possibility of presenting technical subject matter in an interesting and thoughtful manner without at the same time sacrificing accuracy. Of the many areas of science perhaps none holds more interest than human genetics. A well-written book concerned with human heredity would not only be an important addition to the growing fund of non-technical science publications but would likely be profitable for the author as well.

A. M. Winchester* has written a book which is "an account of everyday human inheritance." In it he has touched on reproduction, modes of inheritance, problems of sex determination and sexual abnormalities, inheritance of blood groups, lethal genes, consanguinity, effects of radiation, nature and nurture, eugenics, and the likelihood of different traits being inherited. That he has not written a good book is to be regretted. The need for a general exposition of human genetics is still present.

Several chapters are very worthwhile. Dr. Winchester's explanation of the inheritance of the A-B-O and Rh blood groups is lucid as is the accompanying description of erythroblastosis. In the discussion of consanguinity the author clearly points out that no harm results from close marriages as such but rather from the increased probability of a person having the same deleterious gene as his "cousin" spouse. These chapters can be read with profit by the layman.

However, other chapters are not so clear. Particularly confusing is the important discussion of mutation. What comprehension is gained by the statement that a "single human gene has a life expectancy of 2,500,000 years without a change"? Is it not clearer and more logical to express mutation rate as the number of mutant genes per normal, or unmutated, genes and to indicate the probability that any given germ cell will contain a newly mutated

**Heredity and Your Life.* A. M. Winchester. Vantage Press, 1956. 333 pp. $5.00.

gene than to say that "one gene of this group is likely to mutate once every 125 years"? Dr. Winchester alludes to this preferred nomenclature when he points out, with respect to chondrodystrophic dwarfs, that the data indicate one mutation for every 11,500 births, or one mutation for each 23,000 parents (why not genes?). But apparently even he is confused by his terminology for on the very next page in a table of human mutation rates the rate for chrondrodystrophic dwarfs is listed as one for every 23,000 *births*.

In the discussion of the importance of Mendel's work there is an unfortunate emphasis placed on the "principle of dominance and recessiveness" as one of the most important principles of heredity. While there is no denying that Mendel coined these terms and indicated their importance it would seem more to the point to emphasize the real significance of Mendel's work, namely, that inheritance was particulate and not "blending" as was previously thought. The author also finds it necessary to coin the term "virgin egg" to distinguish the unfertilized haploid egg from the fertilized diploid egg. Would not the term zygote have been suitable here and, while on the subject of terminology, such words as homozygous, heterozygous, genotype and phenotype? Surely these are not difficult words to understand. The use of some of these terms might have prevented the error on page 103 of speaking of "nine different gene combinations" when nine different phenotypes was intended. It is not clear to this reviewer how even nine phenotypes could be correct if one is dealing with four heterozygous gene pairs showing *intermediate* inheritance. Only dominance could give this result. Another feature of the book which is distressing to the reviewer is the oversimplification of the complexity of human inheritance. For example, the first clear mention of incomplete penetrance is not to be found until page 287. Also the selection of illustrative pedigrees with perfect ratios (p. 198 and 206) seems misleading although the author has made clear in other places that 3:1 ratios are only probabilities.

One further point should be mentioned. In the discussion of radiation and mutation Dr. Winchester correctly points out that mutation rate varies linearly with dose and that there seems to be no threshold effect. From this he concludes that the testing of atomic devices, by adding to the naturally occurring radioactivity, will increase the number of deleterious mutations and therefore the number of defective children born in the next and subsequent generations. With this statement the reviewer is in complete agreement and certainly these are facts that prey upon our conscience. But in these times of heated political discussion should it not also be pointed out that at the present level of fall-out such an increase will be statistically undetectable and that there seems no likelihood that the human race will be wiped out by such testings?

Certain minor errors have crept into the book. A split infinitive is found (p. 20). The date for the first explosion of an atomic bomb is incorrect (p. 234). The text states that in figure 11 (p. 104) will be found the proportions of different skin colors resulting from the marriage of two mulattoes. No such proportions are seen. On page 208 is the oft-repeated statement that Charles Darwin and Abraham Lincoln were each the result of a cousin marriage. This is not correct.

To make this book palatable for the layman the author has included many "interesting" facts and anecdotes. Indeed, he found the story of Rasputin so intriguing that it was necessary to include it twice (p. 144 and 214). In his preface Dr. Winchester says that this book was written for the bride and groom, for adolescent boys and girls, and for mature people. This is the principal shortcoming of the book. In his attempt to write for everyone the author has produced a book satisfactory for no one.

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EVIDENCE FOR A NEW ALLELE AT THE *W*-LOCUS OF THE MOUSE

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AN understanding of pleiotropic effects at a single locus may be greatly enhanced with the appearance of new and qualitatively different alleles. However, when mutations occur in animals of different genotype, it is difficult to distinguish between new alleles and new expressions of previously described genes. The purpose of this paper is (1) to describe briefly a new mutation, W^v in the mouse, with effects upon erythrocyte level, germ-cell number, and coat pigmentation; (2) to present evidence establishing this as a member of the *W*-series; and (3) to demonstrate its difference from previously described mutations at this locus.

Description of the New Mutant

The new mutant was found by Dr. George E. Jay in his C3H colony at the National Institutes of Health and was characterized by extensive white spotting on the ventrum with some white on the back, particularly on the crown of the head. Dr. Jay established the inheritance of the spotting pattern as a dominant and noted that crosses between spotted individuals occasionally resulted in black-eyed white offspring which died before weaning age. The mutant was offered to us for further analysis. In our hands, crosses between spotted C3H/Jay individuals gave the following results: 20.8 percent (59/284) of the offspring were black-eyed whites, obviously anemic, with an average survival time of 8.2 days (0-18). Blood counts on thirteen 0-1 day old anemics gave a mean of 1.04 ($\pm$.04) $\times$ 10^6 RBC/mm^3 compared with a mean of 4.58 ($\pm$.24) $\times$ 10^6 RBC/mm^3 for 14 normal littermates. No attempt was made to distinguish homozygous and heterozygous newborns. When the hair-coat appeared, the presumed heterozygous offspring from these crosses showed large areas of white, similar to Jay's description. No diminution of pigment intensity was grossly visible in the colored areas of the coat. At 7 to 8 weeks, in 11 littermate spotted/normal pairs, there was no significant difference ($P = 0.7$) between the erythrocyte numbers of heterozygotes (mean 11.27 $\times$ 10^6 RBC/mm^3) and homozygous normals (mean 11.46 $\times$ 10^6 RBC/mm^3), nor in their mean cell volumes (hematocrit mean, 47.2 percent for heterozygotes, 47.5 percent for homozygous normals). Histological sections of the testis of a newborn anemic male showed it almost completely devoid of spermatogenic tissue. Independent studies[4] indicate that embryos homozygous for this mutant exhibit a deficiency in germ-cell number as early as the ninth day.

These observations demonstrate that the new mutant, when homozygous, results in a severe anemia, leading to death shortly after birth, an absence of melanocytes, and a disturbance in the early differentiation of germ cells. When heterozygous, the mutant appears to have no discernible effect upon erythrocyte number or size in adults, but does lead to extensive white-spotting, at least on its original genetic background.

Tests of Allelism with Known Genes

The observed effects of the new mutation upon erythrocyte number and size, pigment distribution, and germ-cell number strongly suggest the possibility of allelism with the *W*-series (*W*, W^v, *w*) or with Steel (*Sl*, *sl*), both of which affect the same triad of characters. Crosses between $W^v w$ individuals from our colony and animals carrying the new mutant yielded 24 percent (12/49) anemic offspring, with an average survival of 3.3 days. Four of these devel-

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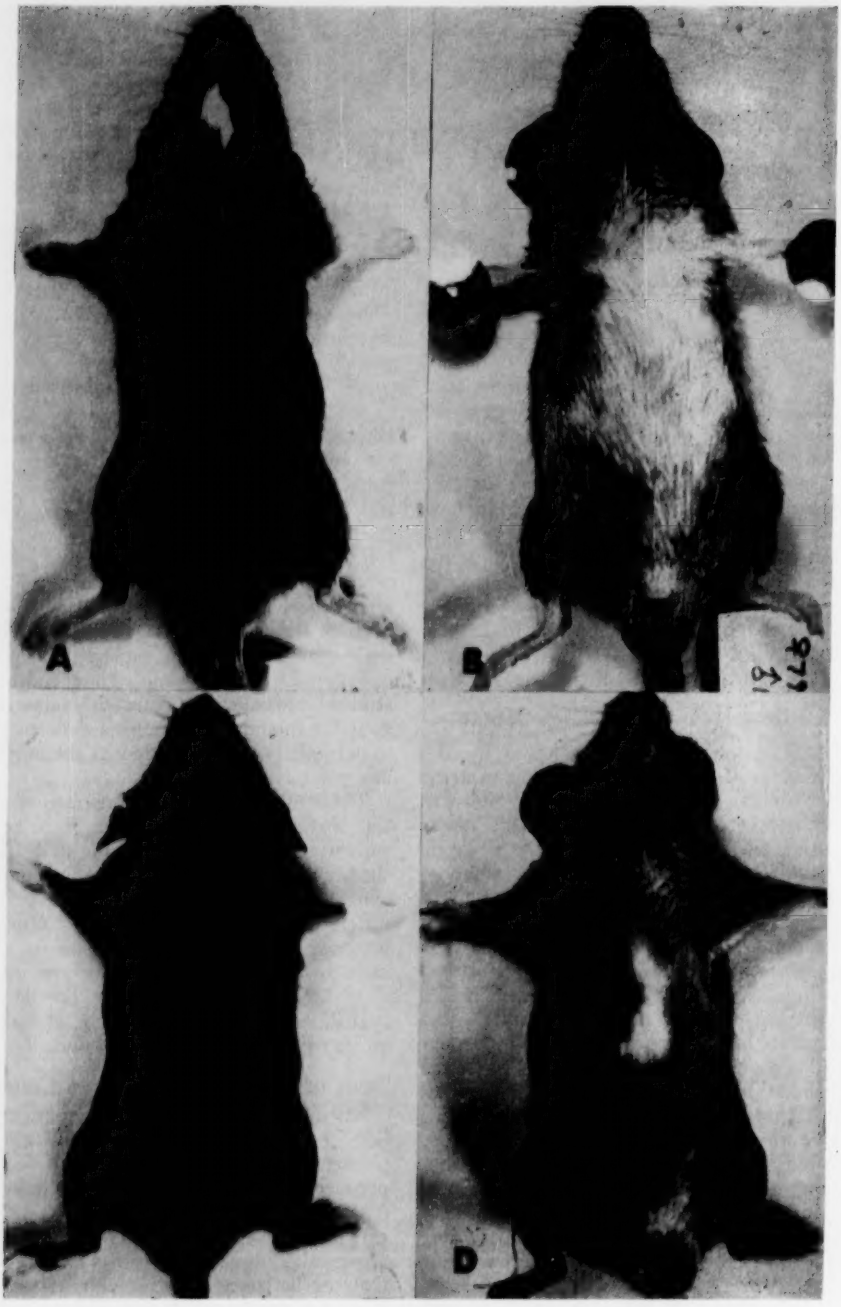

DORSAL AND VENTRAL ASPECTS OF WHITE SPOTTING

Figure 7

A—Dorsal aspect of *AaWiw* male (group A) showing white spotting on head, feet, and small spots on back. *B*—Ventral aspect of *AaWiw* male (same individual as in *A*) showing large white spot covering 35 percent of the ventral surface. *C*—Dorsal aspect of *AaWw* male (group D) showing very small amount of white spotting, restricted to tips of feet and a fleck on the crown of the head. *D*—Ventral aspect of *AaWiw* (same individual as in *C*) showing small white spot covering 4 percent of the ventral surface.

oped far enough to develop a hair coat, which was completely white. Crosses between $W^v w$ individuals, isogenic with C57BL/6, and animals carrying the new mutant yielded 18 percent (14/77) black-eyed whites, of which six survived to adulthood. Crosses between heterozygous *Sl sl* animals, isogenic with C3H, and animals carrying the new mutant yielded no anemic all-white individuals among 63 offspring. These results, indicating the new gene is a member of the *W*-series, led to its designation as W^j, the superscript referring to its discovery by George Jay.

Comparison of W^j with previous *W*-locus Mutations

The observation in $W^j w$ animals of white on the dorsum and very large areas of white on the ventrum brings into question the relationship of the new mutation to the previously studied *W*-alleles, *W* and W^v. The locus appears to be relatively mutable, and more than one instance of mutation to each allele has been observed.

The original *W*-mutation of the fancy has been studied in our laboratory on a background isogenic with C57BL/6 (30 backcross generations) and in four inbred strains (WB, WC, WH, WK) developed here in a selection-inbreeding program to develop types favoring survival of the *WW*-homozygote[9]. On all five of these backgrounds, dorsal spotting in *Ww* heterozygotes is extremely rare, and the belly spots are moderate in size. In all cases, the blood counts and mean erythrocyte volumes of *Ww* adults have not differed significantly from those of their *ww* littermates[5]. *WW* individuals have always been extremely anemic, usually lethal shortly after birth, with survival time ranging from a mean of two days in C57BL/6 to 11.6 days in the WC-strain[9].

An independent mutation to a *W*-allele occurred in the C3H/Jax colony in 1952, and on this C3H background the new mutation, called W^x, could not be distinguished in heterozygous or homozygous effect from the original *W*. In C3H/Jax-$W^x w$ individuals, dorsal white spots have not been observed, and ventral spots are moderate in size.

Two independent W^v mutations have also been studied in our laboratory. The original W^v mutation occurred at the Jackson Laboratory in the C57BL colony of Dr. A. M. Cloudman[2]. This has been studied both on its original background and in combination with many other color genotypes[5]. An independent mutation, indistinguishable from W^v, occurred in 1945 in the C57BR colony, and was studied on this background until its loss in 1947. With both W^v mutations the pigment of the $W^v w$ heterozygote was significantly less intense than that of *ww* littermates. Although the extent of white-spotting was greater than in *Ww* animals otherwise of the same genotype[7], dorsal spotting was rare. In all genetic backgrounds investigated to date, $W^v w$ individuals have had significantly lower erythrocyte counts, with significantly higher mean cell volumes, than have their littermates[5]. $W^v W^v$ individuals develop into sterile black-eyed white adults, most of them living a complete life-span[7].

On most genetic backgrounds, approximately one-half of the WW^v individuals survive to adulthood, and are sterile black-eyed whites. All sterile severely anemic types (WW, WW^v, $W^v W^v$) show a deficiency in number of primordial germ cells in 9 to 12 day old embryos[3].

Thus with all previous mutations at this locus, *Ww* individuals have had normal erythrocyte number and mean cell volume, full pigment intensity, and relatively small belly spots, while *WW* individuals have been postnatal lethals, black-eyed white and sterile. All $W^v w$ individuals have had reduced erythrocyte number, increased mean erythrocyte volume, reduced pigment intensity, and somewhat larger belly spots, while $W^v W^v$ individuals have been viable sterile black-eyed whites.

In all respects except amount of white spotting, the new mutation, W^j, resembles *W* much more than it does W^v.

Critical Evidence for Difference in Spotting Effect of *W* and *W^j*

The critical question is whether the source of the observed difference from *W* in the amount of white spotting lies in the point mutation itself, or in the particular C3H subline, C3H/Jay, in which the mutation occurred. It is conceivable that Jay's C3H subline, separated since 1949 from C3H/Jax, may have accumulated spotting modifiers sufficient to account for the observed differences in amount of white spotting. Hence comparison of the amount of white in genetically comparable *Ww* and *W^jw* individuals was essential.

This was accomplished by means of reciprocal crosses between C3H/Jay with and without *W^j* and our WC-strain with and without *W*. Two groups of animals (A and B), carrying the *W^j* allele in the C3H/Jay × WC F_1 hybrid background, and two other groups, (C and D) carrying the *W* allele in the same genetic background, were produced. The use of reciprocal matings (A vs. B, C vs. D) made it possible to look for maternal effects.

Percentages of white on the dorsal and ventral surfaces of 5 to 6 week old offspring of these four types were determined as follows. The mice were killed with chloroform, placed in a standardized position, with fore and hind limbs outstretched, beneath an Exa 35 mm. miniature reflex camera, and photographed from dorsal and ventral aspects (Figure 7*A-D*). Projection drawings were made of prints enlarged to a standard size. The total area of white spotting was determined on each with a compensating polar planimeter (Table I).

Although the numbers are small, inspection of the data suggests a significant difference between the proportions of white with the two mutant alleles. Analysis of variance confirmed this, with a probability of less than .001 that the differences among the four mating groups could be due to chance. The source of this difference was identified further by a series of t-tests of differences between groups (Table II).

It is clear that the alleles *W* and *W^j* produce significantly different amounts of white spotting. All of the groups with *W^jw* (A, B, A+B) have significantly more ventral white than the comparable groups with *Ww* (D, C, C+D). In one case there is also a significant difference, in this small sample, in amount of dorsal white (A vs. D).

In addition, a separate analysis of incidence of white spots on the crown of the head showed a significant difference between the two genotypes in frequency and size of these spots. In the study of spot distribution, the dorsal aspect of the head was divided into four unit areas, and incidence of white in each unit recorded. Each of the 13 A group and 15 B group animals showed a white spot on the crown of the head, with an average of 2.6 ± .3 units containing white in A animals, and of 3.1 ± .2 units in the B animals. Only one of the nine C+D animals showed white on the head, in one area only, giving a mean for these types of 0.1 ± .1 units of white. Incidence and size of head spot is thus significantly higher on the same genetic background in *W^jw* than in *Ww* animals.

These data, though limited in extent, are nevertheless sufficient to indicate that *W* and *W^j*, on the same genetic background, differ significantly in their white-spotting effect. Hence *W^j* is a new and qualitatively different allele in the *W*-series.

TABLE I. Comparison of percentage of dorsal and ventral white in *Ww* and *W^jw* mice otherwise genetically comparable

Group	Mating	No.	Dorsal white mean	Dorsal white range	Ventral white mean	Ventral white range
A	WC-*ww* ♀ × C3H/Jay-*W^jw* ♂	13	3.1%	0.6-5.6%	29.8%	16.7-50.0%
B	C3H/Jay-*W^jw* ♀ × WC-*ww* ♂	15	8.6%	1.7-26.3%	34.3%	13.0-58.7%
C	C3H/Jay-*ww* ♀ × WC-*Ww* ♂	3	0.4%	0.3-0.6%	6.2%	2.2-11.0%
D	WC-*Ww* ♀ × C3H/Jay-*ww* ♂	6	0.9%	0.3-2.3%	3.7%	2.3-7.6%

TABLE II. Results of t-tests identifying significant differences in percent of white-spotting on dorsal and ventral surfaces in each of the mating groups

Groups compared	d.f.	Genotypes	Dorsum t-value	Dorsum probability	Ventrum t-value	Ventrum probability
B vs. A	27	*W^jw/W^jw*	1.29	.2<P<.3	0.98	.3<P<.4
B vs. C	17	*W^jw/Ww*	1.06	.3<P<.4	3.65	P<.01
D vs. C	8	*Ww/Ww*	0.06	P>.5	0.42	P>.5
A vs. D	18	*W^jw/Ww*	4.37	P<.001	4.34	P<.001
			Dorsum and ventrum combined			
A + B vs. C + D	74	*W^jw/Ww*	t	Prob.		
			5.0	P<.001		

Summary

A new mutation, *Wi*, is described and established as an allele of the *W*-series, with effects similar to those of *W*, except for the amount of white spotting observed.

Critical evidence is given that this white-spotting is due to the mutation itself, and not to the genetic background.

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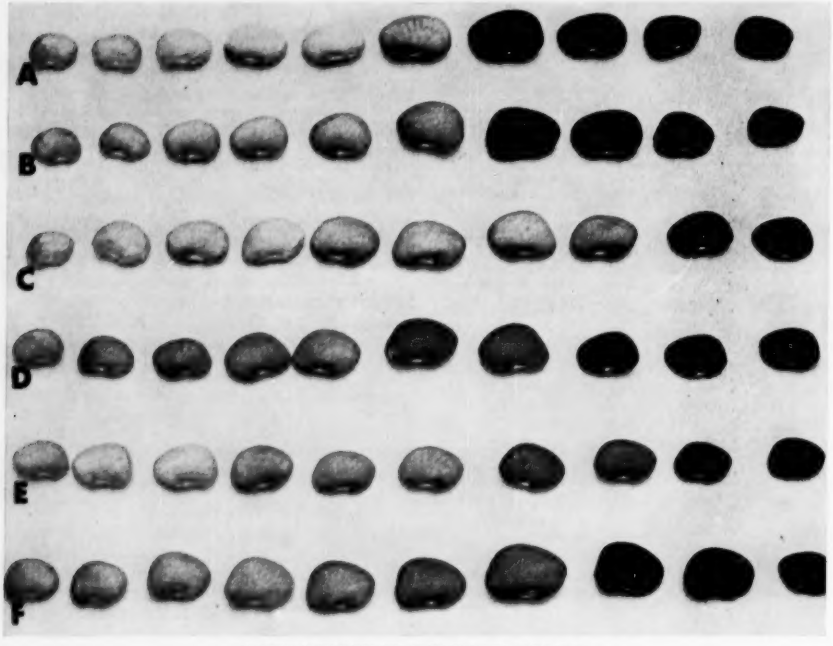
INHERITANCE OF A BASE SEED-COAT COLOR FACTOR IN LIMA BEANS

W. P. Bemis*



DEVELOPMENT OF BASE COLOR

Figure 8

The development of the base color in immature lima beans of the black, purple and red color classes. The development progresses from left to right. *A*—Blue base black; *B*—Red base black; *C*—Blue base purple; *D*—Red base purple; *E*—Blue base red; and *F*—Red base red.

THE inheritance of the five uniform seed-coat colors, black, dark red, purple, red, and white in lima beans has been reported by Allard[1] to be governed by the interaction of three independent gene pairs. The dominant gene *C* acts in an epistatic manner as a basic color factor in the production of colored flowers and seed-coat colors and governs the production of red seed-coat color. In the presence of *C*—, the dominant gene *R* governs the production of dark red seed-coat color, the dominant gene *P* governs the production of purple seed-coat color and purple flower color, and the combination of dominant genes *R* and *P* governs the production of black seed-coat color and purple flower color. The variability among individuals in the color groups studied by Allard suggests the existence of some seed-coat color genes less effective than the ones he described. A black seed-coat plant used as a source of breeding material in the lima bean breeding program at the University of Illinois was found to give segregation of a fourth gene for the expression of seed-coat color.

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Genetics of Base Color Gene *B*

The F_2 population from the cross Green-Seeded Henderson (white seed-coat, white flower) × Illinois Accession 1532 (black seed-coat, purple flower) was grown in 1955, and a base color gene was found to be segregating independently of the three-seed-coat color genes described by Allard. The symbol *B*, *b* is proposed to represent this base color gene. The observed segregation of seed-coat color, base color, and flower color are shown in Table I. The phenotypic description of the seed-coat colors are the same as described by Allard, with the exception of the addition of the term blue or red base preceding the color designation. The interaction of the *C*, *R* and *P* genes in the expression of seed-coat and flower color confirm the work of Allard. Additional crosses were made, and the F_2 progenies were grown in 1956. The parental types and observed segregations for seed-coat color genes are shown in Table II. The parental types in Table II were limited to the purple and red color classes as it is in these two classes that the effect of the base color can be detected in the mature seed. The data in Tables I and II indicate the base color gene *B* is independent of genes *C*, *R* and *P*. The dominant gene *B* in the presence of *C*— governs the development of a blue base color prior to color development governed by the *P* and *R* genes.

Additional data from the F_2 population used in Table I indicated coupling linkage between the gene *B* and a factor for green cotyledons (*G*). The observed and expected parental and recombination types are shown in Table III. The map distance between genes *B* and *G* is 30 ± 3.7 units, calculated from the formulas and tables published by Allard[2].

Description of Base Color Phenotypes

The development of color in the maturing lima bean is shown in Figure 8. The development of the red base color early in the maturation of the lima bean is presumably due to the action of the *C* gene. The dominant *B* gene in the presence of *C*— inhibits the early

TABLE I. Observed segregation of seed-coat color, base color, and flower color and goodness of fit to ratios of 81: 27: 27: 9: 27: 9: 9: 3: 64, 3: 1, and 9: 7, respectively, in the F_2 of the hybrid green-seeded Henderson × Illinois Accession 1532

Phenotype			Number	Number
Seed-coat	Flower	Genotype	observed	expected
Blue base black	purple	*C*—*R*—*P*—*B*—	94	100.9
Red base black	purple	*C*—*R*—*P*—*bb*	41	33.6
Blue base dark red	white	*C*—*R*—*ppB*—	30	33.6
Red base dark red	white	*C*—*R*—*ppbb*	12	11.2
Blue base purple	purple	*C*—*rrP*—*B*—	43	33.6
Red base purple	purple	*C*—*rrP*—*bb*	13	11.2
Blue base red	white	*C*—*rrppB*—	7	11.2
Red base red	white	*C*—*rrppbb*	2	3.7
White	white	*cc*———	77	79.7
Total			319	318.7
χ^2 — 6.3644				
P — 0.30-0.50				
Blue Base		*B*—	174	181.5
Red base		*bb*	68	60.5
			242	242.0
χ^2 — 1.0799				
P — 0.30				
Purple flower		*C*— *P*—	191	179.4
White flower		*cc* or *pp*	128	139.6
			319	319.0
χ^2 — 1.5694				
P — 0.20-0.30				

development of the red color and governs the later development of a blue base color (Figure 8). These two base colors are easily distinguished in the immature stage of development of the lima bean seed. In Figure 8 the fifth or sixth bean from the left in each row clearly shows this difference. The base color becomes masked by the black and dark red seed-coat colors as the seed matures to the dry bean stage. The purple and red seed-coat colors are modified by the base color. Visual color readings following the Munsell system for the purple and red seed-coat colors with different base colors are shown in Table IV.

The Munsell color designations in Table IV represent the average reading for each seed-coat color class. There were variations within each color class, but the differences between the color classes were great enough to permit separation into distinct classes. The production of purple flower color indicating the presence of the *P* gene was used as a check in classifying seed-coat color of the red base purple phenotype.

TABLE II. Observed segregation of seed-coat color and goodness of fit to various monogenic, digenic and trigenic F_2 ratios for five different parental type crosses

Parental lines	F_2 Segregating Populations			
	Phenotype	Genotype	Observed	Expected
Blue base Purple (*CCrrPPBB*)	Blue base Purple	*C_rrP_B_*	71	71.3
	Red base Purple	*C_rrP_bb*	31	23.8
×	Blue base Red	*C_rrppB_*	18	23.8
White	Red base Red	*C_rrppbb*	4	7.9
(*ccrrppbb*)	White	*cc*———	45	42.2
F_2 Seg. — 27:9:9:3:16	$\chi^2 = 4.828$		169	169
	P = .30-.50			
Blue base Purple (*CCrrPPBB*)	Blue base Purple	*CCrrP_B_*	85	91.7
	Red base Purple	*CCrrP_bb*	31	30.6
×				
Red base Red	Blue base Red	*CCrrppB_*	35	30.6
(*CCrrppbb*)	Red base Red	*CCrrppbb*	12	10.2
F_2 Seg. — 9:3:3:1	$\chi^2 = 1.087$		163	163.1
	P = exceeds .70			
Blue base Red (*CCrrppBB*)	Blue base Red	*C_rrppB_*	74	86.6
	Red base Red	*C_rrppbb*	31	28.9
×				
White	White	*cc*———	49	38.5
F_2 Seg. — 9:3:4	$\chi^2 = 4.376$		154	154
	P = .05-.10			
Blue base Purple (*CCrrPPBB*)	Blue base Purple	*CCrrPPB_*	50	49.5
×				
Red base Purple	Red base Purple	*CCrrPPbb*	16	16.5
(*CCrrPPbb*)				
F_2 Seg. — 3:1	$\chi^2 = .020$		66	66
	P = exceeds .80			
Blue base Red (*CCrrppBB*)	Blue base Red	*CCrrppB_*	85	84.75
×				
Red base Red	Red base Red	*CCrrppbb*	28	28.25
(*CCrrppbb*)				
F_2 Seg. — 3:1	$\chi^2 = .003$		113	113
	P = exceeds .95			

TABLE III. Parental and recombination genotypes in the F_2 population indicating coupling linkage

	Parental		Recombinations		
Genotypes	*B/G*	*b/g*	*B/g*	*b/G*	$\chi^2 = 26.380$
Observed	151	29	23	39	P = less than .01
Expected	136.1	15.1	45.4	45.4	% crossing-over 30 ± 3.7

Summary

Base color gene *B* when in the presence of gene *C* inhibits the red base color and governs the production of a blue base color in immature lima beans. The gene *B* was found to be segregating independently of the *C*, *R*, and *P* genes. Linkage between gene *B* and a green cotyledon factor, was calculated to be 30 ± 3.7 map units. The purple and red seed-coat color classes of dried seed were modified by the presence of different base colors.

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TABLE IV. Seed-coat color designations of four phenotypic color classes according to the Munsell system, showing the effect of different base colors on the seed-coat color of the dried bean

Phenotypic Color Class	Munsell Color Designation
Blue base Purple	7.5 P 5/2
Red base Purple	10.0 RP 3/2
Blue base Red	2.5 R 4/4
Red base Red	5.0 R 2/6

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LAND OF THE MOON-CHILDREN

THOSE who know Clyde Keeler can expect a most interesting and vivid story, well illustrated and skillfully written. This book* fulfills these expectations admirably. Perhaps the most important function of the book is that of preserving a record of many of the details of the Panama Cuna Indians' culture. Like so many other cultures of non-literate peoples the day is fast approaching when little will be left of the elaborate rituals and beliefs of the tribe. Their genes will last much longer than their gods. This is fortunate. There is little to commend such practices as exorcising devils and believing in dreams. The folly of such superstitions is well illustrated by the following bit from the book.

* * *

One day a woman came to see the chief of Ailigandi shortly after dawn.

"I had the most horrible dream," she said.

"What is it?"

"I dreamed that the chickens on this island have a disease that will spread to our children and kill them all!"

"Horrible," chorused the town fathers. "This woman has dreamed that all of our children are going to die because of the chickens, so the chickens must all be killed at once!"

The boys of the town rushed out with big sticks and in a matter of minutes some one hundred and fifty dead chickens were floating in the sea.

* * *

Only the tenth and last chapter has much to do with the moon-children. They are slightly shorter and lighter in weight than normal Cuna Indian children. Their skin in general lacks all pigment and is horribly blistered by short exposures to sunshine, to which it also responds by producing scattered blotches of pigment as in true albinos. Head hair at birth is usually white, changing to a rich golden in youth, and then to a light brown or even reddish brown in middle life.

The eye color is a rich blue, a much deeper blue than seen in recognized albinos of the white race. Moon-child eyes are weak and lateral nystagmus was present at all intensities of light under which they were examined —even down to the dim twilight of the Cuna hut (0.8 to 1.6 foot candles). The metabolic rates of the moon-children seem to be reduced and their hands are often cold and clammy. The evidence seems to be sufficient that the moon-child represents not merely a pigment variation but rather a pleiotropic syndrome affecting morphology, physiology and psychosomatic behavior.

After two years of collecting, Dr. Keeler

**Land of the Moon-Children*. Clyde E. Keeler. University of Georgia Press, Athens, Ga. $4.50. 1956.

had records of 152 living moon-children. His adjustments of the 1950 census led him to consider the total Cuna Indian population to be 20,234 persons. The reviewer considers this to be an incidence of 0.75 percent, not 1.75 percent as given on page 200 of the book. Regardless of whose arithmetic is correct, this gives a gene frequency of at least 8.7 percent, meaning that one out of 12 persons carries the recessive albinistic gene concerned. This last is probably not too valid an estimate as the gene is probably concentrated in particular sections of the tribe as a result of the recognized inbreeding. This is interesting at any rate because, as Dr. Keeler points out, the moon-children were in abundant supply according to records of 1681 and thereafter. Considering the fact that eugenic pressure has been exerted against the moon-children, resulting in the early liquidation of many of them, it is remarkable that there are still so many carriers of the gene in the population. The author thinks that the heterozygotes are often lighter colored than the normal homozygotes. The reviewer suggests that the heterozygotes also have some reproductive advantage and thus offset the counter-selection against the homozygous moon-children. Proof of this would be valuable.

In view of the thesis of the author that the moon-child gene is also prevalent among Mayas, Aztecs and Navajos, it would be well if he would devote more attention to the problem of gene frequency in his Cuna pedigrees as related to surviving children and family size. Something most exciting might come from his efforts.

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ABOUT MICE AND MAN

AT this time when so much effort is being exerted toward the stimulation of young students into scientific research, high school science teachers will be grateful for this introduction to mammalian biology* written for the precollegiate student. The book reflects the author's long experience with young science students at Saint Mark's School, at the Secondary Summer School of the Roscoe B. Jackson Memorial Laboratory, and at the Precollegiate Science Summer School of the Worcester Foundation for Experimental Biology.

The approach presented is the proper one. The author first teaches the student how to care for mice properly, emphasizing to the student that the first requisite for a biological research project is a healthy research animal. He follows this with a discussion of the life cycle of the mouse preparing and encouraging the student to breed and raise his own experimental animals. In the next section on the anatomy of the mouse one begins particularly to appreciate the author's general approach. Instead of starting the student at the top with a project such as the study of changes induced by radiation, he teaches the student the normal anatomy of the mouse while at the same time stimulating the student to look for variations from the normal by pointing out certain ones that have been observed and that would be interesting for further study. Following this the author teaches the student simple procedures that can be used in many research projects in biology. These include techniques for simple blood and urine analyses, following the estrous cycle, transplantation of tumors, and then more complicated techniques such as for transplantation of fertilized ova. Simple laboratory equipment is discussed with emphasis on how the student can build the equipment rather than encouraging him to buy it already constructed. A source of supply is usually given for materials that must be purchased. The student is next introduced to techniques in surgery of the mouse. He is taught how to do such operations as gonadectomy, splenectomy, adrenalectomy, and transplantation of ovary to spleen or to the ovarian capsule in another mouse. The author explains the type of studies for which each operation can be used and suggests interesting new studies waiting to be done. This is followed by a chapter on how to study the effect of lowered oxygen supply on the intrauterine development of mice, a field in which the author has been particularly interested for a number of years. Next the student is taught how to perfuse organs of the mouse, and the book is concluded with a list of reference books with comments on the particular value of each.

The book is paper bound and is apparently not intended as a final edition. In further editions some of the anatomical drawings might be improved.

Throughout the text the author's approach is that of starting with the known and, from the periphery of the known, reaching out into the unknown. Many high school students today choose their little research projects so far out in the unknown for them that they are hopelessly lost from the outset. With this book these energetic youngsters could be started out with the proper approach to a lifetime in productive biologic research.

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**About Mice and Man; an Introduction to Mammalian Biology.* Frederick R. Avis. 194 pp., J. Weston Walch, Publisher, Portland, Maine, 1957. $3.00.

THE CURLY AND ANGORA CHARACTERS IN THE RANCH MINK

RICHARD M. SHACKELFORD*

COLOR has traditionally been among the important qualities of the fur animal pelage, but during the past two decades it has come to be the most important single attribute of the ranch raised mink (*Mustela vison* sp.). As a consequence, commercial producers have paid little attention to the appearance of other fur characters and even less to their propagation.

Several fur abnormalities have occurred in a number of mink herds, two of the more interesting being the "curly" and "angora" characters. Neither of these aberrancies appear at the present writing to offer promise of positive value for the fur trade: indeed, the certainty that they are of negative value prompted two practical breeders to donate the original breeding stocks to this laboratory for genetical analysis. Notwithstanding, at least one breeder believes that long fur in the mink may possibly find a place, especially since it can be produced in all color phases seen in fur of standard length. In pursuit of this belief, angora is being combined with several color phases, and with certain other fur characters.

Curly

Hereditary variations in hair texture and form were among the first characters to be observed and reported in laboratory forms and pet stocks. As early as 1905 Castle[2] discussed such characters in the guinea pig and rabbit, and by 1940 Castle and King[3] included two genes for curly (*Cu1* and *Cu2*) and one for kinky (*k*) in their linkage studies of the rat. Grüneberg[4] lists three genes (*wa-1, wa-2* and *Ca*) having phenotypically similar effect in producing wavy and caracul conditions in the mouse.

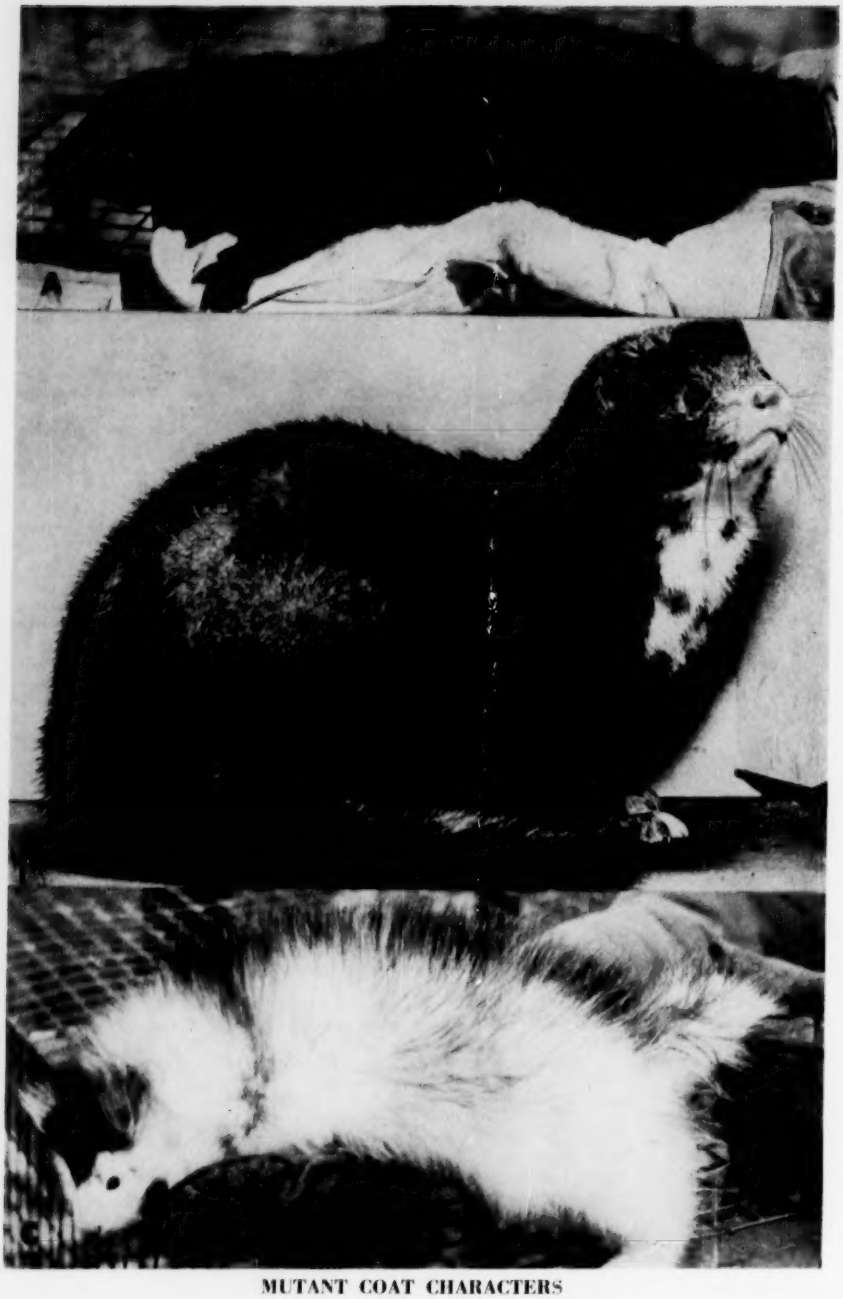
All curly mink at this station are decendants of a curly male obtained from a Minnesota ranch in 1947. This male and a curly sister had been born in a litter of five the previous year. The other three kits and both parents were reported to be normal, and thus it was assumed that possibly a single recessive gene was responsible for this variation from the normal smooth coat. However, curly × normal matings produced curly and normal offspring in essentially equal numbers from the outset, so one of the parents of the original male must have been a curly; the following description of the variation in expression of the character suggests a reason for the probable error in classification by the owner.

The breeding results with curly mink is summarized in Table I. In 28 litters from curly (*Cycy*) × normal (*cycy*), 38 curly and 45 normal young have been obtained which does not deviate significantly from a 1:1 ratio. 16 curly to 9 normal kits resulted from curly (*Cycy*) × curly (*Cycy*) matings, approximating a 3:1 ratio. Only those offspring surviving for final classification at maturity of the adult coat (November) have been tabulated.

It is clear that this curly character in the mink is brought about by the presence of a gene in heterozygous condition which may be symbolized *Cy*. It is doubtful, however, that *Cy* is completely dominant to its allele. Several of the curly kits from curly (*Cycy*) × curly (*Cycy*) matings had few or no guard hairs over the body proper although the extremities showed the normal number of exceedingly curly guard hairs: a male and a female of this type (presumably *CyCy*) have been bred to normal coated individuals for two seasons and have produced only curly offspring to date.

Curly mink derived from curly × unselected normal matings vary greatly in the degree to which the character is expressed. The least affected individuals have only a few slightly curly guard hairs down the center of the back, while at the other extreme are those having the guard hairs over the entire body so curly that there is a tendency for tight ringlets to be formed (Figure 9*A*). Most curly mink fall between these two extremes of expression, and unless the most severely affected ones are observed it will be difficult to understand the reason for terming the character curly. In the mildly aberrant form, the guard hairs stand up straight rather than flow backward as in the normal animal (Figure 9*B*); this gives the appearance of a rough coat, and the impression is found to be correct on stroking the fur. Some mink of the *Cycy* genotype appear practically normal to the casual observer which

*Genetics Department, University of Wisconsin and Agent, Fur Farm Investigations, Agricultural Research Service, U. S. Department of Agriculture. Paper number 651 from the Department of Genetics, University of Wisconsin. Published with the approval of the Director of the Station.



MUTANT COAT CHARACTERS

Figure 9

A—Curly (*Cycy*). This individual shows the extreme form of the character; guard hairs over the entire body are so much curled that tight ringlets tend to form. *B*—Normal or "Wild Type." This mink happens to be of the Bluefrost (*Ff*) color phase. *C*—Angora (*ll*). The guard hairs are two and one-half to three times standard length, and the underfur nearly twice as long as that of the normal mink, note long tufts of hair in and around the ears. This individual is the Black Cross (*Ss*) color phase.

leads to the supposition that curly may be more commonly distributed in ranch bred populations than is generally realized.

The individual guard hairs of curly mink are curved downward in the portion that extends beyond the underfur, and may also be slightly rotated on the axis; the underfur seems to be unaffected. It may be difficult or impossible to distinguish with complete accuracy between kits of a litter segregating for curly and non-curly by inspection of the juvenile fur, and final classification must be delayed until the animal develops its first adult coat; the degree of curliness remains constant throughout life thereafter. The vibrissae are normal in curly mink as contrasted to the curled vibrissae of most of the waved, kinky and caracul phenotypes in laboratory forms.

Angora

Castle[1] early reported investigations concerning the inheritance of the "Angora" character in mammals. Today, unusually long hair in domestic species is commonly seen in the cat—angora or persian—and not infrequently one sees or reads of the angora rabbit. Only one farm has reported angora mink (Figure 9C) to the writers knowledge; although an occasional long furred individual has been born among the standard dark mink of normal coat length for several years on this Wisconsin ranch, it was not until the 1952 breeding season that angora mink were available for tests in this laboratory.

Table II consists of a summary of the breeding results with the angora mink. Only offspring with fur of normal length resulted from 13 matings of angora × normal; from 17 matings of angora × normal with one angora parent, 23 angora and 30 normal kits were produced which is a fairly close approximation of the 1:1 ratio; four matings of angora × angora gave 12 young, all angora. Thus the angora character results from homozygosity for a recessive gene in this species, and *l* is suggested as an appropriate symbol.

Casual observation only is enough to reveal that the fur of the angora is much longer than that considered normal for domestic mink or the numerous subspecies of *Mustela vison* found in the wild: the guard hairs are from two and one-half to three times standard length, and the underfur nearly twice as long as its counterpart in the average mink. The most striking quality of the angora to those accustomed to viewing mink in general is the exceptionally long tufts of hairs in and around the ears which give the appearance of rather large ears; this has led most observers to comment that these mink look more like angora cats than mink.

Young mink are born essentially naked, and several weeks pass before the angora character is detectable; the appearance of abnormally long hair on the ears and toes gives the first indication that an individual is destined to develop the angora coat. Fur length varies among angoras, but from the mature juvenile

TABLE I. Breeding results of curly mink

No. Matings	Parents		Offspring	
	Curly (*Cycy*) × Normal (*cycy*)		Curly	Normal
20	♂♂	♀♀	29	41
8	♀♀	♂♂	7	6
Observed			38	45
Expected (1:1)			41.5	41.5
9	Curly (*Cycy*) × Curly (*Cycy*)		16	9
Expected (3:1)			18.75	6.25

TABLE II. Breeding results of angora mink

No. Matings	Parents		Offspring	
	Angora (*ll*) × Normal (*LL*)		Angora	Normal
8	♂♂	♀♀	0	33
5	♀♀	♂♂	0	15
Observed			0	48
Expected (0:1)			0	48
17	Angora (*ll*) × Normal (*Ll*)		23	30
Expected (1:1)			26.5	26.5
4	Angora (*ll*) × Angora (*ll*)		12	0
Expected (1:0)			12	0

coat onward there is no difficulty in classifying the offspring into normal and angora in segregating litters. The guard hairs of all angoras appear sparse as compared to the normal, but this may well prove to be an optical illusion.

Summary

The curly and angora mutant characters in the ranch mink (*Mustela vison* sp.) have been described. Breeding data have been presented which indicate clearly that mink of the genotype *Cycy* exhibit the curly phenotype, and that homozygosity for the gene *l* results in the angora coat.

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Corrigendum

In Figure 10 of the paper: "Brachytelephalangy and Brachymesophalangy in the Same Family," which was published in this *Journal* (48: 21-25. 1957), there is an error which should be corrected as follows: Individuals II-17, III-18, III-32 should be marked with a diagonal line as they are dead or not tested. The calculated ratios are in accordance with this data only.

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